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INSIDE SCIENCE

The Vital Story of Vitamin B$_1$

(Thiamine)

by Science Writer

History. The discovery of vitamin B_1 resulted from research into the cause of beriberi. Almost 50 years passed between Eijkman's discovery of the relationship of the disease to diet and the famous work of Jansen and Donath who first isolated the crystalline vitamin from rice bran.

Within ten years of that first isolation the vitamin's chemical structure was determined and it was successfully synthesized.

Eijkman's work resulted in the development of a theory that beriberi was caused by a lack of some factor in the diet and not by a toxin or infectious agent. This idea was not readily accepted until the growth of dietary knowledge proved it correct.

Isolation and Synthesis. In 1926 Profs. Jansen and Donath accomplished the isolation of crystalline vitamin B_1 from rice bran. In 1931 Windaus and coworkers successfully isolated pure vitamin B_1 and established its empirical formula. In 1936 R. R. Williams, and independently R. Grewe, explained the vitamin's chemical structure. That year, R. R. Williams and J. K. Cline accomplished the synthesis of thiamine which is in wide use today. Andersag and Westphal also synthesized the vitamin in 1936. Another synthesis was described by Bergel and Todd in 1937.

Photomicrograph of B_1 crystals

Chemical and Physical Properties. Thiamine hydrochloride is white, water soluble, with a nut-like, salty taste and yeast-like odor. Its empirical formula is: $C_{12}H_{17}ClN_4OS \cdot HCl$. Thiamine produced by synthesis is identical chemically and in biological activity with that obtained in pure form from nature.

Deficiencies. A deficiency of thiamine is characterized by these symptoms: depression, irritability, fearfulness, lack of initiative and interest, loss of appetite. Symptoms vary since in usual practice deficiencies of other water-soluble vitamins occur. Medical treatment is simple: a sufficient amount of thiamine is administered to relieve symptoms quickly and the physician provides for a continuing adequate intake.

Beriberi victim

A severe deficiency of thiamine leads to beriberi, a serious and sometimes fatal disease. While beriberi is almost a medical curiosity in the United States, it is common in countries in which polished white rice is a staple of the diet.

Human Nutrition Requirements. Thiamine is one of the nutritive elements the human body needs daily and does not store in quantity. The minimum daily requirements established by the U. S. Food and Drug Administration for the prevention of symptoms of thiamine deficiency disease are:

Adults ....1.00 mg.	Children (1-5 incl.)..0.50 mg.
Infants ....0.25 mg.	Children (6-11 incl.).0.75 mg.

The Food and Nutrition Board of the National Research Council recommends the following dietary intake of thiamine for healthy persons in the U. S. A.

Recommended Daily Intake in Milligrams

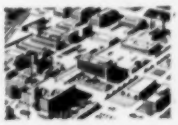
Age	*Men*	*Women*
25	1.6	1.2
45	1.5	1.1
65	1.3	1.0
Pregnant (3rd trimester)		1.5
Lactating		1.5

The Council recommendations for infants and children vary below and above these figures, based on age and sex. Various illnesses and stress situations can exhaust vital reserves of thiamine. So, for the physician, vitamin B_1 is prepared in various dosage forms and potencies for therapeutic and prophylactic use.

How do human beings receive thiamine? It is widely distributed in foods of animal and vegetable origin, particularly cereal grains and dry legumes. Because of public demand for refined products which millers must meet for obvious economic reasons, a loss of thiamine and other factors occurs during processing. The thiamine loss is overcome through the use of *enrichment* in cereal grain products for which Federal Standards exist, or in other foods such as breakfast cereals, by *fortification* or *restoration*. When enriching, fortifying or restoring, the food processor adds the necessary amount of pure thiamine (and other vitamins and minerals) to the food so that the finished product meets Federal, state and territorial requirements or contributes to the consumer an amount of the vitamin which dietary experts believe significantly useful.

Thiamine is extensively used for the enrichment of cereal grain foods such as white flour, white bread and rolls, macaroni products, farina, corn grits and meal, milled white rice. The story of these uses is delightfully told in a separate brochure which is available on request for reference or educational purposes.

Production. Huge production facilities at the Hoffmann-La Roche plant in Nutley, New Jersey, deliver highest quality thiamine *by the tons*. Roche manufactures thiamine hydrochloride and thiamine mononitrate. These fine products, which equal or exceed U.S.P. specifications, are ideal for use by pharmaceutical makers and food processors. Years of experience in research and manufacture have made Roche the leader in vitamins.



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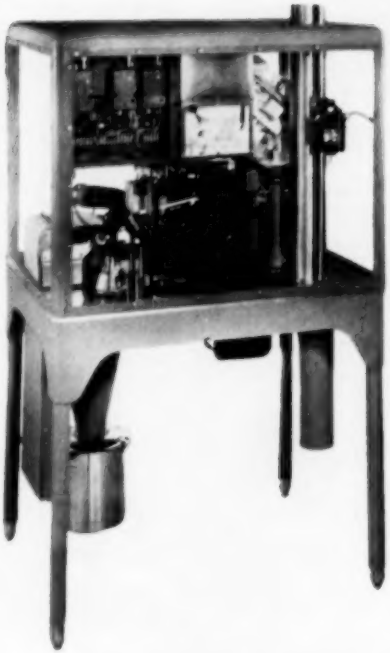

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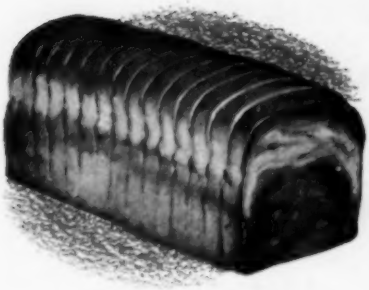

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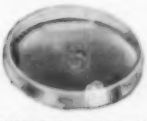

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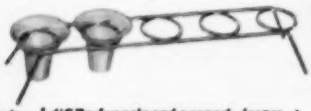

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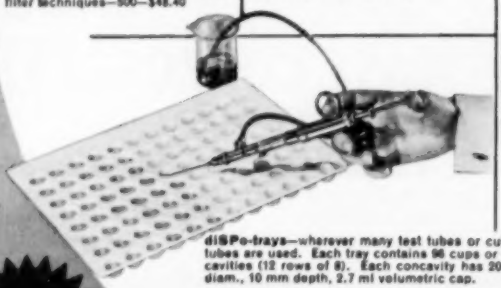

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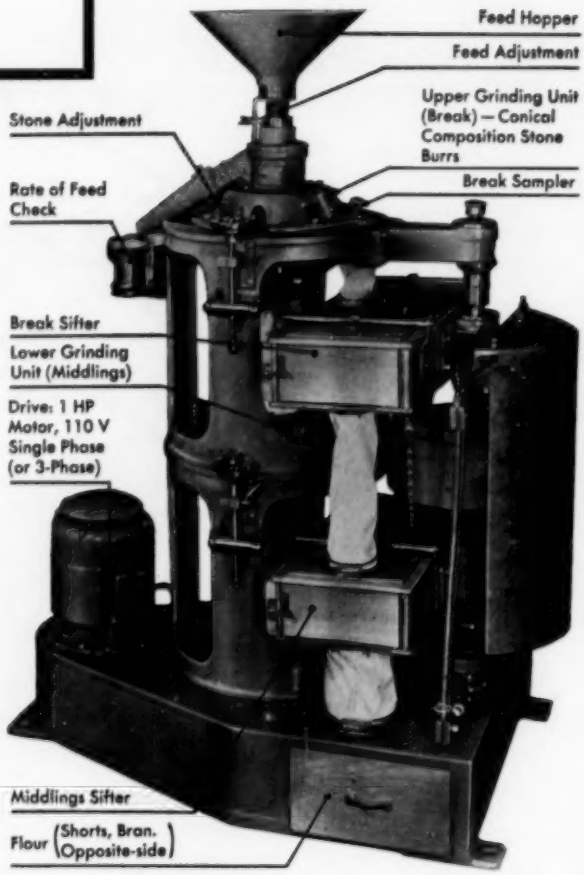

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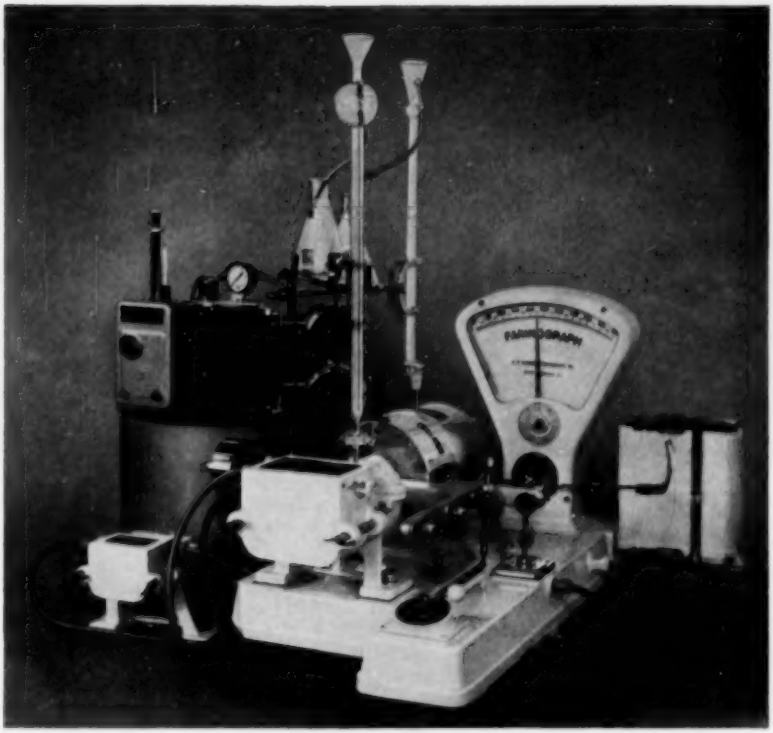

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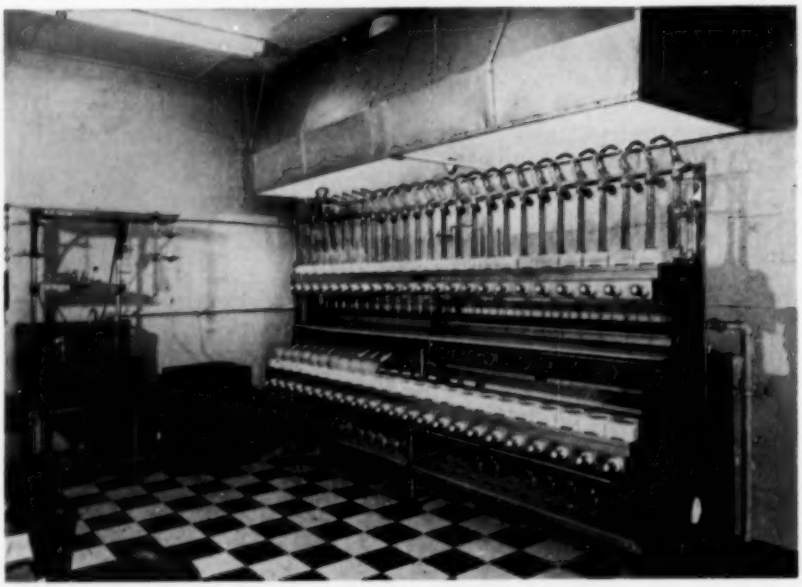

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CEREAL CHEMISTRY

VOL. 34 MARCH, 1957 NO. 2

SORPTION OF WATER VAPOR ON WHEAT FLOUR, STARCH, AND GLUTEN[1]

W. BUSHUK[2] AND C. A. WINKLER[3]

ABSTRACT

Adsorption-desorption isotherms for water vapor on flour, starch, and freeze-dried and spray-dried gluten have been obtained at 27°C. using a McBain-Bakr sorption balance in conjunction with a high-vacuum technique. Characteristic sigmoid isotherms showing linear hysteresis were obtained in the relative pressure range to 0.85 for the four adsorbents. The hysteresis loops were quite reproducible and the sorption was completely reversible if the relative pressure of 0.9 was not exceeded. At higher relative pressures the flour and the glutens were physically altered and the sorption was no longer reversible. At lower pressures, considerable caking was observed in flour and gluten, but not in starch. Apparently, this caking did not have any effect on subsequent adsorption on the sample. Adsorption of water vapor by flour appears to be a bulk property of the material and not dependent on particle size. A 24-hour heat treatment of the flour at 100°C. in a vacuum reduced its sorptive capacity for water vapor by about 20%. The effect of temperature on adsorption of water vapor was similar for flour, starch, and gluten; the effect was greatest at low adsorption (low relative pressures) and became almost negligible as the saturation pressure was approached. For low adsorptions, the isosteric heat of adsorption for the three materials was considerably higher than the heat of condensation but decreased gradually as the amount adsorbed increased, until at about 16% to 20% moisture content the isosteric heat was essentially equal to the heat of condensation.

A reasonably good fit of the Brunauer, Emmett, and Teller (B.E.T.) equation was obtained for the flour-water isotherm if the number of adsorbed layers at saturation pressure was assumed to be five or six. The B.E.T. area was 235 sq. m./g., and the B.E.T. heat of adsorption was 12.6 kcal./mole.

Water appears to be almost unique among liquids in its ability to form doughs from flour. The commercial uses of flour, accordingly, depend almost entirely upon this important property of water. The interaction of water with flour has been studied by a number of techniques (8, 9). Although considerable progress has been made, the specific mechanism of the cross-linking that leads to the formation of the three-dimensional structure of doughs is still unknown.

[1] Manuscript received June 27, 1956. Contribution from the Physical Chemistry Laboratory, McGill University, Montreal. Based in part on a thesis submitted by W. Bushuk to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the Ph.D. degree, May, 1956.

[2] Grain Research Laboratory, Winnipeg, Manitoba. Holder of National Research Council of Canada Studentship and Fellowship at McGill University.

[3] Department of Chemistry, McGill University.

The present sorption studies were undertaken to obtain more information about the interaction of water with flour or specifically with starch and with flour proteins. Sorption of water by flour has been studied previously (12); however, there has been almost no attempt to account for the sorptive capacity of flour on the basis of its physical and chemical structure.

For the present investigation, sorption studies with water vapor were of prime concern. Various aspects of water sorption on flour, and on starch and gluten prepared from the same flour are described in this paper. In addition to the studies reported here on sorption of water, vapor-phase sorption studies have been made with a number of organic liquids that do not form doughs, for purposes of comparison; a subsequent paper deals with this aspect of the investigation.

Materials and Methods

Flour. The following flours were used in the course of this investigation: Flour A, used for the major portion of this investigation, was an unbleached, improver-free, straight-run sample commercially milled from high-grade Canadian hard red spring wheat. The protein content ($N \times 5.7$) of this flour was 15.0%.

Flour B, used to study the effect of particle size, was an unbleached, improver-free, straight-run sample, milled on a laboratory mill from a pure variety (Selkirk) of Canadian hard red spring wheat. Its protein content was 15.3%. A portion of this flour was fractionated on a Rotap sifter into fractions of different particle size. The fractions held on (H.O.) and passing through (P.T.) the various sieves and their protein contents were as follows:

Fraction	*Protein Content* %
H.O. No. 120	15.0
H.O. No. 140	15.2
H.O. No. 170	15.4
H.O. No. 200	16.2
P.T. No. 200	15.8

Flour C, used to study the effect of heat treatment, was an unbleached, improver-free, straight-run sample, milled from a blend of Canadian hard red spring wheat of the 1954 crop. Its protein content was 14.8%. Prior to sorption experiments, this flour was treated as follows: Approximately 50 g. of flour were placed into a series of small glass vials which were evacuated and sealed. These were then held at 100°C. for various periods of time. Moisture content of the flour before the heat treatment was about 1.5%.

Starch. The starch was separated from a portion of flour A. Ap-

proximately 200 g. of flour were made into a stiff dough and the starch was washed out by working the dough under a slow stream of water. To the resulting suspension, enough sodium chloride was added to make the solution approximately 2 *N* in the salt. This was allowed to stand for several hours to dissolve the salt-soluble proteins. The starch was separated by centrifugation, washed several times with ethyl alcohol to remove the alcohol-soluble proteins, and dried in a vacuum at 65°C. It was stored in a desiccator over silica gel.

Gluten. Two different samples of gluten were used: (a) gluten separated from flour A and dried by the freeze-drying process (13); and (b) gluten separated from the same flour and dried by the spray-drying process (14). The freeze-dried gluten had a protein content of 69.8% and the spray-dried sample had a protein content of 86.6%.

To obtain uniform adsorbent samples, a single milling of flour (or a single preparation of gluten) was stored in such a way as to minimize the changes that might occur during storage. In this study, 5-g. samples of flour (or gluten) were stored at low temperatures (about 4°C.) in 25-ml. round-bottomed flasks which were sealed after a 4-hour evacuation. A fresh sample was used for each new experiment.

Apparatus. Figure 1 is a schematic diagram of the apparatus. The

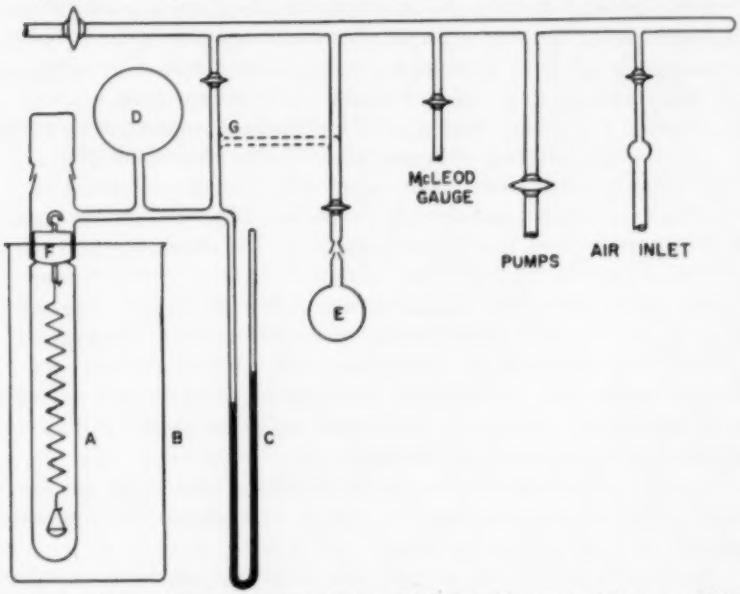

Fig. 1. Schematic diagram of apparatus showing: The McBain-Bakr sorption balance suspended in adsorption tube *A*, a portion of which is immersed in the water bath, *B*; a mercury manometer, *C*; a 3-liter bulb, *D*, to increase the volume of the adsorption chamber; a flask, *E*, which contains the vapor source; and auxiliary equipment used in high-vacuum technique.

sorption chamber consisted of the following: An adsorption tube, *A*, constructed from 38-mm. Pyrex tubing to which was connected a mercury manometer of 10-mm. Pyrex tubing; a 3-liter bulb, *D*, to increase the total volume of the adsorption chamber (this could also be used as a vapor storage bulb); and a bulb, *E*, for storing the sorbate liquid.

The sorption chamber was connected to a manifold which included standard equipment used in high-vacuum techniques. High vacuum was obtained by a mercury diffusion pump backed by an oil pump. Low pressures were measured with a McLeod Gauge.

McBain-Bakr sorption balances were used to measure the extent of sorption. Two quartz spirals,[4] of sensitivities 1.80 and 1.72 mg. per mm. of extension, were used. These were suspended in the adsorption tube, *A*, from a hook attached to a glass bulb, *F*. The bulb was supported by a constriction in the adsorption tube. The portion of the adsorption tube, *A*, that surrounded the quartz spiral and the adsorbent sample was immersed in a water bath, *B*.

The apparatus used to study the effect of temperature was, in principle, the same as that described above, except it was constructed in such a way that the complete adsorption chamber was immersed in a thermostatically controlled water bath.

Experimental Techniques. A sample of adsorbent (about 100 mg.) was conditioned, prior to adsorption of water vapor, by a 12- to 14-hour evacuation at 27°C. at maximum pressure of 10^{-4} mm. The weight of the adsorbent became constant usually after about 6 to 8 hours of evacuation. The initial conditioning was made at this low temperature to preserve the original characteristics of the adsorbents. The term "dry" adsorbent, as used in this paper, refers to the condition of the adsorbent after this conditioning treatment. The moisture content of flour conditioned in this manner was generally about 0.7% as determined by the A.A.C.C. air-oven method (1). No attempt was made to obtain the same moisture content by the two methods, since this would have required much more drastic initial treatment (higher temperatures) and would probably have altered the physical characteristics of the adsorbent. Starch and gluten, which initially had much lower moisture contents than flour, showed a behavior similar to that of flour during the initial conditioning treatment.

Sulfuric acid solutions and pure water were used as the sources of vapor. Prior to each experiment, these were degassed by repeated freezing, evacuation, and melting.

After the initial conditioning of the adsorbent, vapor was admitted to the adsorption chamber and the equilibrium adsorption was deter-

[4] Obtained from Houston Technical Laboratories, Houston, Texas.

mined from the extension of the spiral measured with a cathetometer. The measurements were then repeated at higher or lower pressures until sufficient data were obtained for a complete isotherm.

This experimental procedure had to be modified slightly to study the effect of temperature on the adsorption process. Instead of determining the complete isotherm at one temperature, it was more practical to determine the equilibrium adsorption at the four temperatures used in this study for a single sulfuric acid solution (approximately constant relative pressure). The temperatures were reproduced by using four thermoregulators set at 20.2°, 30.1°, 40.8°, and 50.2°C. The adsorption measurements were then repeated using sulfuric acid solutions of progressively lower concentration.

Corrections for the effect of buoyancy on the sample and the container were less than the experimental error. Blank experiments showed that there was no adsorption on the spiral or the empty sample container until a relative pressure of 0.9 was exceeded.

Results and Discussion

Rate of Adsorption. Kinetic studies of the adsorption process showed that, in the absence of interfering gases that are not adsorbed on flour to any measurable extent at 27°C., the rate of adsorption of water vapor on flour, starch, and the two types of gluten was quite rapid, and complete equilibrium was established in less than 4 hours. The adsorption was about 75% complete in 15 minutes after the sample was exposed to the vapor. There is some indication that the rate data could be satisfactorily treated by a first-order plot.

The rate of adsorption was not affected by varying the size of the adsorbent sample approximately twofold, by packing it more compactly, or by varying the relative pressure of the sorbate; however, the presence of slight amounts of air in the adsorption chamber decreased the rate of adsorption very markedly. This evidence suggests that the measured rate of adsorption was diffusion-controlled.

Sorption Isotherms. Previous studies of sorption isotherms for water and flour (3) were obtained, with sulfuric acid solutions as the source of constant vapor pressure. In this study, it was decided to use pure water as the vapor source to make the results for water strictly comparable to those obtained for the organic vapors. It was therefore of interest to compare the results using the two methods for varying the vapor pressure, since it has been postulated that they might be different under certain conditions (15). Isotherms obtained by the two methods for one sample of flour were almost identical. Accordingly, it may be concluded that, in the apparatus used in this study, the two

methods give comparable results and therefore either method could be used without introduction of an additional source of error.

The sorption isotherms at 27°C. using pure water as the vapor source for flour, starch, and freeze-dried and spray-dried gluten are shown in Fig. 2. These isotherms are plotted in the standard manner, namely, the amount adsorbed (X in mg. per g. "dry" adsorbent) is plotted as a function of the relative pressure, P/Po, where P is the equilibrium pressure and Po is the vapor pressure of pure water at 27°C.

The isotherms for flour and starch are similar to those reported previously (3, 16). Vapor-phase sorption studies on freeze-dried and spray-dried gluten have not previously been reported. For the four materials, the isotherms are sigmoid in shape showing considerable

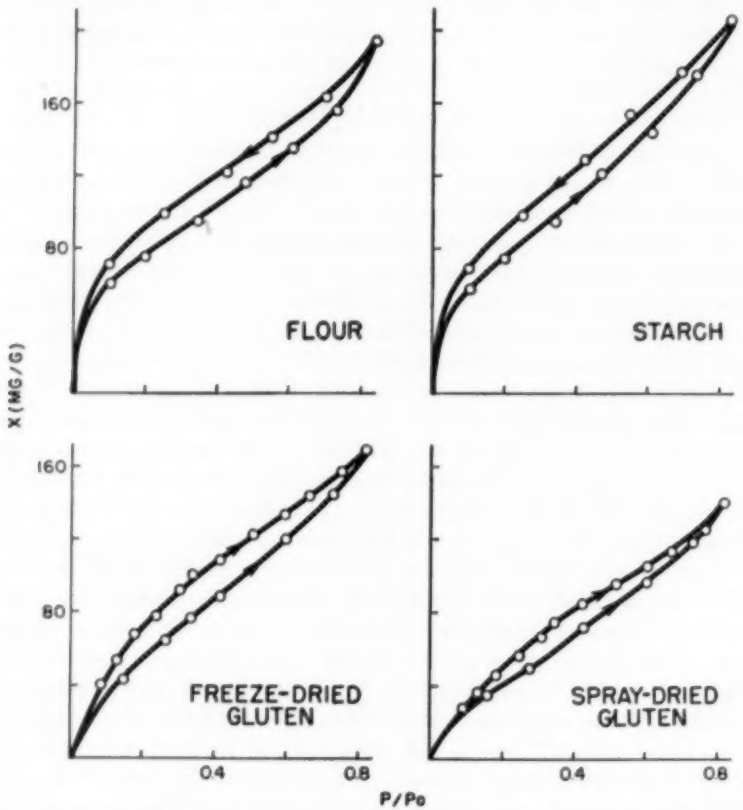

Fig. 2. Adsorption-desorption isotherms at 27° C. showing the amount of moisture (X) as a function of the relative vapor pressure (P/Po).

hysteresis between the adsorption and the desorption branches, which persists over almost the entire pressure range investigated. The sigmoid shape of the isotherm and the hysteresis effect seem to be characteristic of natural high polymers such as proteins (18), starch (16), and cellulose (10).

Comparison of the isotherms for the four materials reveals that the sorptive capacity of the four materials for water vapor show the following trend: Starch > flour > freeze-dried gluten > spray-dried gluten. These differences probably reflect real differences in the ability of these materials to bind water. The difference in sorptive capacity of the two gluten samples suggests that the spray-dried sample might be slightly denatured.

Reproducibility of Hysteresis Loop. The reproducibility of the hysteresis loop was examined by carrying out two successive adsorption-desorption cycles with water on flour. The results obtained in the range of relative pressure 0 to 0.85 showed that the hysteresis effect is reproducible within the limits of experimental error.

Considerable caking was observed in flour and gluten, but not in starch, when water vapor was adsorbed at relative pressures 0.8 to 0.9. This is assumed to be incipient doughing. At still higher relative pressures, the degree of adsorption is sufficient to completely and permanently alter the physical nature of the flour or gluten, and this renders sorption irreproducible by decreasing the amount of adsorption in the second cycle. This change in the adsorbent does not seem to affect the hysteresis loop.

Water vapor adsorbed below 0.9 relative pressure could be completely removed from the adsorbents by about 6 to 8 hours of evacuation. Similar results with flour were obtained by Babbitt (3). However, if the adsorption occurred at a relative pressure above 0.9, then complete removal of the adsorbed water is practically impossible; there appears to be a certain amount of irreversibly bound water on the flour. This could not be accurately measured with available McBain-Bakr balances.

Effect of Particle Size and Heat Treatment on Sorptive Capacity of Flour. A study of the effect of particle size on the extent of adsorption is desirable since this provides a means of varying the external surface area. If the adsorption is of Van der Waals type, then it would be expected that the extent of adsorption would vary directly with the specific surface area.

Adsorption isotherm data were accordingly obtained for a sample of flour and five different particle-size fractions obtained from it. A representative part of the data is plotted in Fig. 3, A, to show the maxi-

mum variation in sorptive capacity. An estimate of the specific surface areas (external) of the various particle-size fractions, determined from sieve sizes, showed that the total variation in surface was about two-fold; however, Fig. 3, A, shows that the extent of adsorption is about the same for all the fractions and the unfractionated flour. It appears, then, that adsorption of water vapor by flour is a bulk property of the adsorbent and apparently independent of particle size. This suggests that adsorption of water vapor on flour is more specific than Van der Waals adsorption.

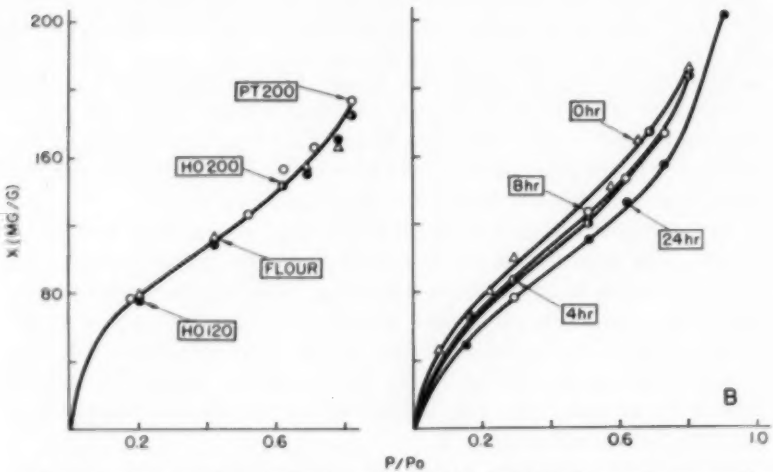

Fig. 3. Isotherms at 27° C. showing the amount adsorbed at various relative vapor pressures for: A. Flour B and various particle size fractions obtained from it. B. Flour C heat-treated at 100° C. in a vacuum.

A study of the effect of heat treatment on the sorptive capacity of flour was of interest for two reasons. First, it seems that heat treatment affects flour quality in a way that suggests that the treatment causes certain components of flour to cross-link[5]; if this is true, then it might be possible to detect some change in the sorptive capacity of heat-treated flour as compared with that of untreated flour. Secondly, there seems to be some disagreement as to the effect of heat treatment on the heat of hydration of flour (17, 19); this relates to the present study since the heat of hydration results directly from the mechanism of the adsorption.

Figure 3, B, compares the adsorption isotherm for normal flour with the isotherms for portions of the same flour that were heat-treated for 4, 8, and 24 hours at 100°C. in a vacuum. The effect of this heat treat-

[5] Irvine, G. N. Private communication.

ment appears to be quite small for short treatments but becomes extensive for much longer treatments. A 24-hour heat treatment reduced the sorptive capacity of the flour by 20%. On the basis of this evidence, it would appear that the heat of hydration for flour (27 cal. per g.) at zero moisture reported by Schrenk *et al.* (17) is probably too low by about 20%. The observations made in the present study are in general accord with the findings of Astbury and Lomax (2). They found, in their studies of heat denaturation of proteins, that during denaturation some of the polar groups interact with each other in such a way as to change a globular type of protein into a more fibrous type.

Effect of Temperature on Adsorption by Flour, Starch, and Freeze-dried Gluten. Adsorption isotherms for flour, starch, and freeze-dried gluten were determined at four temperatures to provide data that could be used to evaluate the isosteric heats of adsorption. The isosteric heat of adsorption is directly related to the energy of interaction between sorbate molecules and the sorption sites. The energy of interaction, in turn, can be suggestive of the mechanism involved.

The sorption data for the three materials at the four temperatures, 20.2°, 30.1°, 40.8°, and 50.2°C., are summarized in Table I.

For each material, the data in Table I yield a family of four isotherms in which the extent of adsorption at constant relative pressure decreases slightly with increasing temperature. The family of isotherms

TABLE I

SORPTION OF WATER VAPOR BY FLOUR, STARCH, AND FREEZE-DRIED GLUTEN AT 20.2°, 30.1°, 40.8°, AND 50.2°C.

	20.2°		30.1°		40.8°		50.2°	
	P/Po[a]	X[b]	P/Po	X	P/Po	X	P/Po	X
Flour	0.12	68.8	0.13	64.3	0.13	59.3	0.15	54.3
	0.23	85.5	0.26	79.9	0.28	76.8	0.31	73.5
	0.43	104.9	0.43	102.8	0.45	99.6	0.47	96.0
	0.62	138.6	0.63	134.9	0.63	131.0	0.64	125.5
	0.76	173.9	0.78	172.3	0.78	168.4	0.79	162.6
	0.89	250.0	0.90	245.9	0.90	241.8	0.90	239.5
Starch	0.10	54.3	0.10	50.5	0.11	46.7	0.12	43.6
	0.30	90.3	0.31	86.4	0.31	83.0	0.32	80.6
	0.48	119.0	0.47	114.8	0.48	110.0	0.48	106.2
	0.66	160.0	0.66	151.9	0.66	140.9	0.67	132.9
	0.75	201.3	0.75	189.7	0.79	182.0	0.76	164.0
	0.92	260.6	0.92	253.8	0.92	247.3	0.91	243.0
Gluten	0.07	34.2	0.09	31.7	0.10	28.8	0.12	26.2
	0.12	51.9	0.13	48.4	0.14	45.0	0.16	39.7
	0.27	71.9	0.27	69.1	0.29	66.2	0.30	63.9
	0.42	88.4	0.44	85.5	0.46	81.6	0.48	76.7
	0.73	151.2	0.74	146.5	0.74	143.2	0.76	139.0
	0.92	257.4	0.92	254.4	0.92	252.1	0.92	249.7

[a] P/Po is the relative vapor pressure.
[b] X is the amount adsorbed in mg. per g. of "dry" adsorbent.

for each adsorbent was displaced according to the relative sorptive capacity of the material. These sorption data were then used in the usual manner to calculate, graphically, the isosteric heat of adsorption for various adsorptions by means of the Clausius-Clapeyron equation. Figure 4 shows the isosteric heats of adsorption for the three adsorbing materials plotted as a function of the amount of water vapor adsorbed. The dashed line represents the average heat of condensation of water. Initially the heats are considerably higher than the heat of condensation and the values decrease with increasing amount of adsorption, approaching the heat of condensation at about 16 to 20% moisture. The three adsorbing materials showed essentially similar behavior.

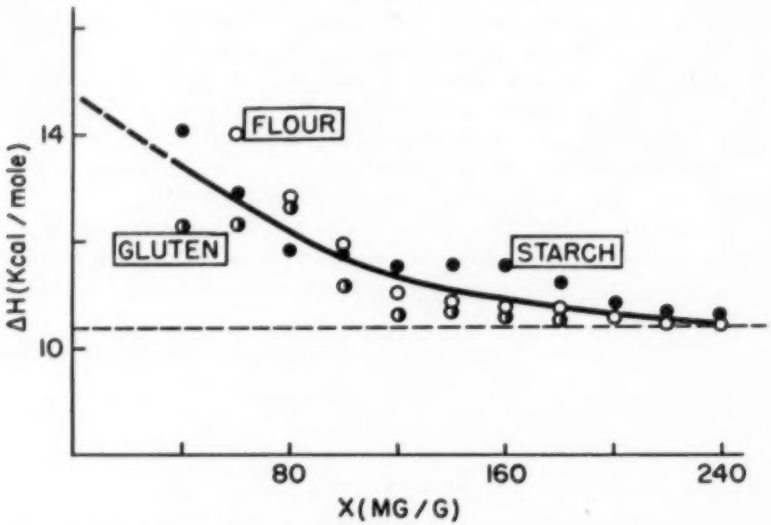

Fig. 4. Variation of isosteric heat of adsorption with increasing water adsorption.

Too much emphasis should not be placed on the actual magnitude of the isosteric heats obtained from the temperature coefficient of adsorption, for two reasons. First, the values include considerable error arising from various graphical manipulations as well as the experimental error in the isotherm data. Secondly, the isosteric heat is a thermodynamic quantity, and as pointed out by Seehof *et al.* (18), application of thermodynamic arguments to isotherms that show a hysteresis effect is almost purely empirical. Accordingly, the values obtained here can only be treated in a qualitative manner.

An isosteric heat greater than the heat of condensation indicates primarily that the energy of interaction between the sorbate and sorp-

tion sites is greater than the energy that holds the sorbate molecules together in liquid state. There is some evidence that this is the case for the systems described in this paper. The value of the isosteric heat that would have thermodynamic significance would be the heat at zero moisture content. The value that was obtained by extrapolation as indicated by the dashed portion of the curve in Fig. 4 was 14.6 kcal. per mole. However, such an extrapolation can give a completely erroneous result, since calorimetric results with other materials (6) have yielded heats that continuously decrease or else increase and then decrease with amount adsorbed.

The decrease in the isosteric heat with amount of vapor adsorbed can be qualitatively explained in a number of ways. First, one hypothesis states that initially, adsorption occurs on the most active sites, giving rise to greatest interaction energy. As these active sites become occupied, adsorption occurs on the less active sites giving lower heats of adsorption. This hypothesis is analogous to that based on the Brunauer, Emmett, Teller (B.E.T.) theory (6), which assumes that the heat of adsorption for the first layer is greater than the heats for subsequent layers.

According to a second hypothesis the decrease in heat of adsorption is due to swelling of the adsorbent. Cassie (7) showed that, in sorption of water vapor by wool which also yields a sigmoid isotherm, the swelling process is highly endothermic and may use as much as 50% of the heat produced by the adsorption process. By analogy, it is suspected that the swelling of the materials used in this study would be endothermic also. Accordingly, to get a true picture of the variation of the isosteric heat with amount adsorbed, the values obtained would have to be corrected for the heat of swelling. Such a correction requires a number of physical constants for the adsorbent which are not yet available for flour, starch, or gluten.

Application of the B.E.T. Theory. The isotherms for the four adsorbents that were used in this study are of sigmoid shape which, according to the B.E.T. theory, are classified as Type II isotherms. The general features of such an isotherm have been described fundamentally by Brunauer (6) and specifically as applied to wheat and flour by Hlynka and Robinson (12) and more recently by Becker and Sallans (4). For purposes of completeness, it might be emphasized that a sigmoid isotherm is characterized by three relatively distinct regions: 1) an initial region concave to the pressure axis, 2) an intermediate almost linear region, and 3) a final region convex to the pressure axis.

To obtain a mathematical representation of such an isotherm, Becker and Sallans (4) preferred to divide the isotherm into three parts

and apply a different equation to each part. In the present investigation, an attempt was made to apply the three-constant B.E.T. equation by the procedure described by Brunauer (6) to the over-all flour-water isotherm.

The values of Xm, the volume of vapor in the first adsorbed layer, and c, a characteristic constant related to the heat of adsorption of the first layer, determined from the linear form of the B.E.T. equation, were found to be 66.2 mg./per g. and 37.7. Using these values and vary-

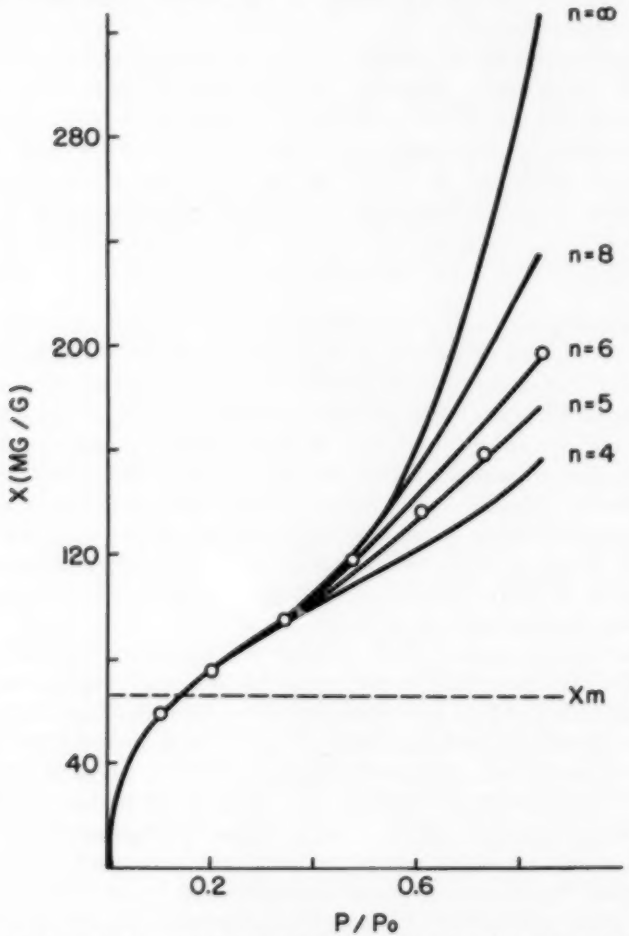

Fig. 5. Isotherms plotted according to the B.E.T. equation using the values of constants Xm and c obtained from the adsorption isotherm for flour shown in Fig. 2 and various n values. Open circles are experimental points and the dashed line is the amount of adsorption in a unimolecular layer according to B.E.T. theory (Xm).

ing the value of n, the number of adsorbed layers at saturation relative pressure, a family of sigmoid isotherms can be obtained as shown in Fig. 5. The open circles represent experimental points. It may be noted that a reasonably good representation of the flour-water isotherm may be had in terms of the B.E.T. equation if n is set equal to 5 or 6.

The most significant factor for the success of the B.E.T. theory is that it provides a means of evaluating the specific surface area of the adsorbent (determined from Xm) and the heat of adsorption for the first layer (determined from c). The specific surface area obtained for the flour used in this study was 235 sq. m. This value is considerably higher than the values (0.16 to 0.60 sq. m.) obtained by the same method from flour-argon isotherms (11). Similar disagreement was observed by Benson *et al.* (5) in the surface areas of a number of proteins determined by the adsorption of water vapor and nitrogen. There is some indication (18) that at least part of the discrepancy might be due to the swelling that occurs during the sorption of water, although it does not seem likely that the increase in surface area owing to swelling would be extensive enough to account for all of this difference. There is also a possibility that a part of the difference in the surface areas might be due to inapplicability of the B.E.T. theory to sorption systems that exhibit a marked hysteresis effect and in which there is extensive swelling of the adsorbing material. Until the reasons for this discrepancy are understood more clearly, it did not seem worth while to apply the B.E.T. equation to any of the other isotherms.

The value of the average heat of adsorption for the first layer of water molecules on the flour used in this study was 12.6 kcal. per mole. Although this value appears reasonable, its magnitude can only be confirmed by a calorimetric determination of the heat adsorption.

No definite conclusions can be made about the sorption mechanism or the role of water in dough formation on the basis of the results presented in this paper. Evidence has been presented, however, which suggests that water interacts with flour components through specific chemical groups, probably by a dipole-dipole type of interaction. Further evidence has been obtained by using sorbate molecules of larger size and smaller polarity. This will be the subject of a later paper.

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SORPTION OF ORGANIC VAPORS ON WHEAT FLOUR AT 27°C.[1]

W. BUSHUK[2] AND C. A. WINKLER[3]

ABSTRACT

The McBain-Bakr sorption balance was used to obtain adsorption-desorption isotherms at 27°C. for methyl alcohol, ethyl alcohol, and carbon tetrachloride vapors on flour. These isotherms are compared with the isotherm for water vapor on flour. The polar sorbates yielded sigmoid isotherms with reproducible hysteresis loops. Carbon tetrachloride gave a linear isotherm with no hysteresis. The relative sorptive capacity of flour showed the following trend in binding these vapors: Water>methyl alcohol>ethyl alcohol>carbon tetrachloride. Apparently the extent of sorption decreases as the molar volume of the sorbate molecule increases. For polar sorbates, the amount of hysteresis, relative to the extent of sorption, appears to increase with molar volume.

Sorption studies were initiated to study the interaction of water with the various components of flour. Previous work (1, 3) has indicated that diffusion may be the main rate-controlling factor in the adsorption of water vapor on flour. If this is the case, then further information on the mechanical aspect of sorption on flour might be obtained by determining the rates of adsorption of sorbates of different molar volume.

Sorption on substances such as proteins that have polar functional groups appears to occur on specific sites, since it seems that only polar sorbates are strongly adsorbed on such substances (2). This evidence suggests that the main attractive force of the sorbent for the sorbate is due to electrostatic attraction of permanent dipoles. It was therefore of interest, from this viewpoint, to study the effect of polarity (dipole moment) of the sorbate on the sorption process.

To study the effects of molar volume and polarity of the sorbate on sorption by flour, comparative studies were made using water, methyl alcohol, and ethyl alcohol as sorbates. Carbon tetrachloride was included in these studies as an example of a nonpolar sorbate.

Studies of the sorption of the lower alcohols on flour are also of particular interest because they do not interact with flour to form doughs similar to those produced with water, although they are similar to water in certain chemical properties.

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[3] Department of Chemistry, McGill University.

This paper summarizes the results of a comparative study of sorption of water, methanol, ethanol, and carbon tetrachloride on flour, and discusses the role of molar volume and polarity in the sorption mechanism.

Materials and Methods

Flour. The flour was an unbleached, improver-free, straight-run sample commercially milled from high-grade Canadian spring wheat. The protein content ($N \times 5.7$) of this flour was 15.0% (dry-matter basis). The same flour was used in sorption of water-vapor studies reported in a companion paper (3).

Methyl Alcohol. Methyl alcohol was the Fisher "Reagent" grade.

Ethyl Alcohol. "Super-dry" alcohol was prepared by the method described by Vogel (5). In accordance with this method, 95% alcohol was refluxed for 6 hours over quicklime and the distillate refluxed for 30 minutes over granulated zinc and subsequently distilled.

Carbon Tetrachloride. Carbon tetrachloride was Fisher "Spectro" grade.

Experimental Procedure. The apparatus and the technique used to obtain the sorption isotherms have been described in detail in the first paper of this series (3). With all sorbates used, the pressure inside the adsorption chamber was varied by admitting a definite amount of vapor from the pure liquid kept under vacuum in a storage flask. The sensitivities of the spirals (McBain-Bakr balances) used to obtain the methyl alcohol isotherms were 1.80 and 1.72 mg/mm. The spirals used to obtain the ethyl alcohol and carbon tetrachloride isotherms had sensitivities of 0.411 and 0.308 mg/mm. Fifty-milligram adsorbent samples were used with the two latter spirals. The results reported in this paper were obtained at 27°C. With all sorbates, adsorption was assumed to be complete when no change in the weight of the adsorbent was observed over a period of at least 2 hours.

Results and Discussion

Rates of Adsorption. Figure 1 compares the rate curves for the adsorption of water, methyl alcohol, and ethyl alcohol vapors on flour at 27°C. The equilibrium relative pressures at which these rate data were obtained were 0.72, 0.72, and 0.70 for water, methyl alcohol, and ethyl alcohol, respectively. Similar rate curves to those shown in Fig. 1 were obtained for each sorbate at other relative pressures.

The rate curves show that, under similar experimental conditions, water is adsorbed on flour much faster than either methyl or ethyl alcohol, and methyl alcohol is adsorbed faster than ethyl alcohol. The

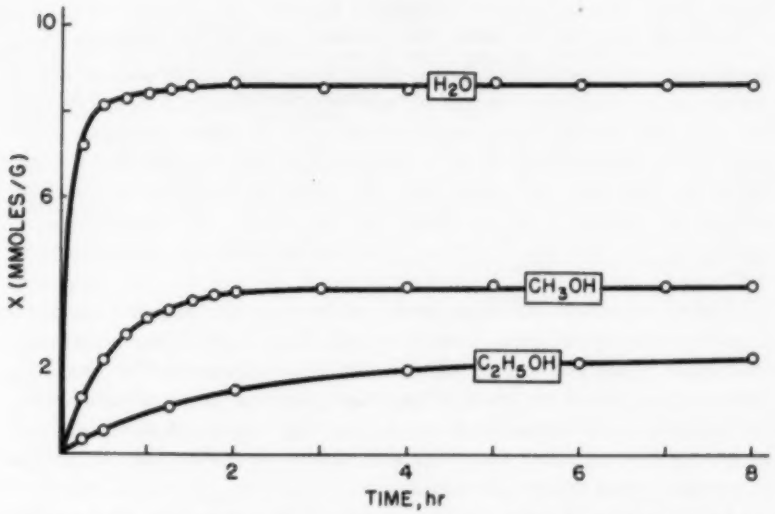

Fig. 1. Rates of adsorption of water, methyl alcohol, and ethyl alcohol vapors on flour at 27°C.

time that was necessary to attain adsorption equilibrium with water was about 3 hours, whereas about 6 hours were necessary to establish equilibrium with methyl alcohol and more than 8 hours (actually about 12 hours) were required to establish equilibrium with ethyl alcohol. The curves in Fig. 1 show also that the equilibrium amounts sorbed, at constant relative pressure, of the three polar sorbates are: water>methyl alcohol>ethyl alcohol.

It was not possible to obtain accurate rate data for adsorption of carbon tetrachloride with the technique used because of the low sorptive capacity of flour for this sorbate.

Previous work (1, 3) indicated that diffusion may be the rate-controlling factor in the adsorption of water vapor by flour. Accordingly, the rate data in Fig. 1 were examined on the basis of diffusion and they fitted reasonably well into the first-order rate equation. The specific rate constant, obtained from the slope of the initial linear portion of the first-order plot, varied inversely with the molar volume. This evidence seems to confirm the previous finding that the rate of adsorption of polar vapors on flour is primarily a diffusion process.

The dipole moments (4) and the molar volumes for the four sorbates used are given in Table I to facilitate discussion of results on the basis of these two physical properties.

TABLE I

DIPOLE MOMENTS AND MOLAR VOLUMES FOR WATER, METHYL ALCOHOL, ETHYL ALCOHOL, AND CARBON TETRACHLORIDE

	DIPOLE MOMENT (DEBYES)	MOLAR VOLUME[a]
		cc.
Water	1.87	18.79
Methyl alcohol	1.69	42.74
Ethyl alcohol	1.70	62.28
Carbon tetrachloride	0.0	103.11

[a] Molecular weight/density at boiling point

A similar inverse relation was found between the amount adsorbed of each of the three polar sorbates used, at constant relative pressure, and their respective molar volumes. It seems apparent that once the sorbate molecules have reached the sorption sites, the number of molecules that can be accommodated within the region of influence surrounding the sorption sites, i.e., the extent of adsorption, would be dependent upon the molar volume.

The Sorption Isotherms. The sorption isotherms, plotted in the standard manner, for the three organic sorbates and water vapor and

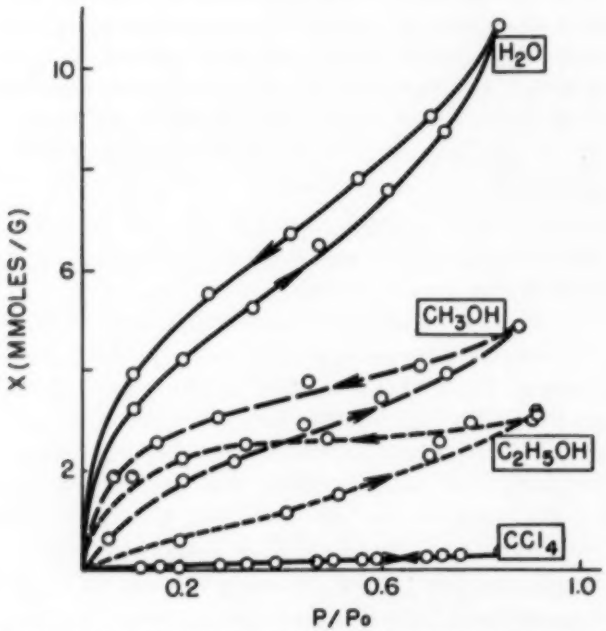

Fig. 2. Adsorption-desorption isotherms for flour at 27°C., showing the amount adsorbed (X) as a function of the relative vapor pressure (P/Po).

flour at 27°C. are given in Fig. 2. The isotherm for water is the same as reported previously (3).

These data show that polar sorbates yield isotherms that are essentially sigmoid in shape, although the isotherm for ethyl alcohol shows only a slight concavity to the pressure axis in the low relative pressure range. In addition to the sigmoid shape, the polar sorbates exhibit the so-called linear hysteresis effect, i.e., extending over the entire relative pressure range. The size of the hysteresis loops shows the following trend: ethyl alcohol>methyl alcohol>water. The isotherms for carbon tetrachloride are essentially linear and exhibit no hysteresis effect. The sorption isotherms show the same trend, as did the rate curves in Fig. 1, in the sorptive capacity of flour for the three polar vapors. On the same basis of comparison, carbon tetrachloride is only slightly adsorbed.

The shape of the carbon tetrachloride isotherm and the absence of a hysteresis loop suggest that a different mechanism probably applies to this sorbate or that the extent of sorption of carbon tetrachloride is too small to show up the characteristics (sigmoid shape and a hysteresis loop) of the isotherms obtained with other sorbates. There is also a possibility that the carbon tetrachloride isotherm might represent a chemical interaction of carbon tetrachloride with the fatty components of the flour rather than sorption.

The data in Fig. 2 substantiate the finding that, for polar sorbates, the molar volume seems to be the prime factor upon which the extent of sorption depends. This effect is particularly demonstrated by the isotherms for the two alcohols. As shown in Table I, these sorbates have essentially the same polarity; accordingly, the decrease in the extent of adsorption seems to be due to increase in molecular size. Similarly, it can be inferred that the increase in the size of the hysteresis loop is probably primarily due to increase in molar volume.

Qualitative Observations and Theoretical Considerations. It was usually observed that flour swelled quite noticeably on taking up methyl or ethyl alcohol vapor. Similar observation was made (3) during the sorption of water vapor. As might be expected, carbon tetrachloride did not produce any swelling that could be observed visually. The vapor-phase caking effect (incipient doughing) obtained with water vapor (3) was not obtained with any of the other sorbates. Unsuccessful attempts were made to break up the flour "cakes," resulting after an adsorption-desorption of water vapor, by adsorbing methyl alcohol onto the caked adsorbent and then desorbing it by evacuation. The cake dispersed readily if it was immersed completely in anhydrous methyl or ethyl alcohol. Higher alcohols and a large variety of other liquid solvents did not show this interaction. The fact that

methyl alcohol, if present in high concentration, can displace adsorbed water (by mass action) is probably the reason why it is a reasonably good solvent for moisture determinations by the Karl Fischer method. Another pertinent observation was that the adsorbed alcohol could not be removed completely by evacuating, for any practical period of time (about 10 hours), as was readily possible in the case of adsorbed water (3). Complete removal of alcohols could be achieved, however, by swelling the flour by sorbing water vapor on the same sample and then desorbing the alcohol and the water by evacuation. Two such treatments were sufficient to remove all the adsorbed methyl alcohol. Both branches of the alcohol isotherms are reproducible after the adsorbed alcohol is completely removed by such treatment.

The main force which causes the adsorption of vapors on flour seems to be the attraction between permanent dipoles. To reach the sorption sites, the sorbate molecules apparently have to diffuse through the flour medium. Accordingly, the rate of adsorption should increase with decreasing molar volume. This was indeed obtained experimentally. The magnitude of the attractive force, i.e., the dipole moment of the sorbate molecule, apparently had no effect on the rate of adsorption that could be observed in these experiments. It seems, therefore, that the polar nature of the sorbate molecule is only a necessary condition for sorption on flour.

The possible hypotheses that will account for the sigmoid shape of the isotherm have been considered in a companion paper (3). The reason for the decrease in the extent of adsorption with increasing molar volume has already been considered. It is therefore necessary only to formulate a hypothesis that will explain the difference in the size of the hysteresis loop for the three polar sorbates. The isotherm for carbon tetrachloride is excluded from this qualitative discussion for reasons previously indicated.

The increase in the hysteresis loop with increasing molar volume follows directly from the assumption that at least part of the hysteresis is due to physical trapping of molecules within the adsorbent. The same degree of shrinkage would produce a greater effect with the larger sorbate molecules.

The shape of the isotherm for ethyl alcohol and flour is quite similar to the isotherm for water and whole wheat (1). This suggests that the low concavity of the adsorption isotherms to the pressure axis, at low relative pressure, may be due to the fact that some of the sorption sites are inaccessible because of the large molar volume of the sorbate in the former case and the physical structure of the sorbent in the latter. The physical obstruction to water molecules is apparently

removed to a certain extent when the wheat kernel is milled into flour.

On the basis of the available evidence, flour contains specific polar groups that interact with the sorbate molecules during the sorption process. Identification of these groups will require further sorption studies with gases such as ammonia and some of its derivatives and hydrogen chloride, which would probably interact with specific types of polar groups because of their basic or acidic nature.

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EFFECT OF BAKING ON THE NUTRITIVE VALUE OF PROTEINS IN WHEAT BREAD WITH AND WITHOUT SUPPLEMENTS OF NONFAT DRY MILK AND OF LYSINE[1]

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ABSTRACT

The protein quality of whole-wheat bread and of the unbaked ingredients containing 0, 3, 6, and 12% nonfat dry milk and 0.166% l-lysine, and of white bread containing 6% nonfat dry milk, was determined by rat-growth studies. Increasing quantities of milk gave increased protein efficiency ratios. The quality of the white bread containing nonfat dry milk was better than that of the whole-wheat water bread and somewhat inferior to that of whole-wheat bread containing 3% nonfat dry milk. Whole-wheat bread containing 0.166% l-lysine, equivalent to the amount of lysine in 6% nonfat dry milk, had a protein efficiency ratio close to that of the bread containing 6% nonfat dry milk, which was not essentially different from that of whole-wheat bread baked without lysine but supplemented by lysine in the diet. The protein efficiency of all of the breads was approximately 20% less than that of their unbaked ingredients. Protein value of bread dried in a dehydrator at temperatures up to 50° C. was not significantly different from that of bread dried at room temperature.

As a result of numerous studies to determine the protein quality of white and whole-wheat breads, it is generally recognized that wheat protein is of poorer quality than animal protein, such as meat, eggs, and milk; that whole-wheat flour and whole-wheat bread are superior to white flour and white bread; and that the wheat protein may be improved by the addition of lysine, milk solids, or other lysine-containing supplements.

While the broad outlines of the picture are clear, the details are by no means complete because the variations in the methods used make comparisons of the studies difficult. For example, in some cases milk was added to flour or ground grain, in others to bread, and in still others milk was incorporated in the bread. In some cases vitamin supplements were used, in others they were not.

While the effect of as much as 24% nonfat dry milk on the nutritive value of white bread has been studied, no more than 6% nonfat dry milk has been included in whole-wheat breads studied. Research has been done on the protein quality of white and of whole-wheat bread and on wheat endosperm and whole wheat; on the effect of supplementing flour and bread with the amino acids that limit the quality of their protein, and on the loss of lysine caused by baking the bread;

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and on the effect of heat on the quality of cereal proteins. Yet no investigation has been made of the effect of fermentation and baking processes used in the preparation of bread on the protein quality of the flour; there has been no comparison of the improvement in the protein quality of the bread afforded by milk and that afforded by an equivalent amount of lysine, nor any evaluation of the nutritional significance of common commercial baking conditions.

Consequently, the purposes of the research here reported have been:

1. To note the effect on protein quality (as shown by rat-growth studies) of the addition of 3, 6, and 12% nonfat dry milk to whole-wheat bread, and to compare the protein qualities of these breads with those of nonfat dry milk and of white bread made with 6% nonfat dry milk.

2. To note whether the proteins of baked bread have the same quality as the proteins of the unbaked ingredients.

3. To determine whether the beneficial effect of 6% nonfat dry milk on the proteins of whole-wheat bread is due principally to the lysine provided by the milk.

4. To determine the extent to which the added lysine is rendered unavailable to the rat by the baking of the bread.

5. To compare the effect on the protein quality of baking whole-wheat bread containing 6% nonfat dry milk at a high temperature for a short time and baking at a moderate temperature for a longer time.

Material and Methods

Diets. Nineteen batches of experimental breads – sixteen from whole-wheat flour and three from white – were baked according to the following formula: flour, 100%; sucrose, 4%; salt, 2%; hydrogenated cottonseed oil, 2%; compressed yeast (or the equivalent of active dry yeast), 2%; nonfat dry milk, 0, 3, 6, or 12%. Four different whole-wheat flours and three patent flours were used in six different experiments, as follows:

Experiment No.	*Flour*	*Protein (14% moisture basis)*
		%
1	Whole-wheat A	13.0
2	Whole-wheat A	13.0
	Patent M	12.2
3	Whole-wheat B	13.3
4 and 5	Whole-wheat C	14.6
	Patent N	13.7
6	Whole-wheat D	15.6
	Patent O	10.7

The nonfat dry milk used was a commercial spray-dried, heat-treated product prepared for the baking industry. The fermentation times em-

ployed in preparing the bread varied with the flour, and most of the breads (1⅓-lb. to 1½-lb. loaves) were baked at 400°–450° F. for 30 or 35 minutes. However, two whole-wheat water breads (G and M, experiments 2 and 3) were baked for 25 minutes at 475° F., and one whole-wheat bread with 6% nonfat dry milk (in experiment 5) was baked at 500° F. for 25 minutes.

The breads were sliced and dried in a dehydrator with circulating air, set at 135° F. The highest temperature attained inside the dehydrator was 122° F. toward the end of the drying period. Drying times were variable. In experiment 6, the breads dried in the dehydrator were compared with breads dried at room temperature. The dried breads were finely ground in a Hobart mill, and refrigerated until used.

The diets were formulated to contain 7 to 10% fat, 4% salt mixture (11), and protein nitrogen as follows: $1.88 \pm 0.02\%$ in experiments 1 and 2; $1.95 \pm 0.02\%$ in experiments 3 and 5; and $2.00 \pm 0.02\%$ in experiment 6, to be supplied solely by the bread being tested. The diets contained from 60 to 85% bread or bread ingredients. The balance of the diets consisted of corn starch.

When the bread ingredients were to be fed unbaked, the dry ones were weighed (without the shortening) and were thoroughly mixed together. Before being added to the mixture, the dry active yeast was killed by heating in an air oven at 82° C. for 30 minutes and was then pulverized. The shortening was melted and blended into the dry ingredients.

The B vitamins were prepared in a 20% ethanol solution. Vitamins A, D, and E were supplied by a mixture, in cottonseed oil, of shark liver oil, irradiated ergosterol, and mixed tocopherols.[2]

Feeding and Experimental Procedure. The animals used were rats of the Long-Evans strain from the stock colony of the Department of Home Economics, weaned when 21 days old or when their body weight reached 40–45 g. The animals were caged separately in wire cages and were fed a basal diet for 2 or 3 days or until they became accustomed to their new environment.

Six experiments were carried out. Although the conditions of the experiments were kept as nearly alike as possible, some variations occurred. The variables were:

[2] Amounts received per animal per day: Supplement A, experiments 1 and 2, thiamine HCl 30γ, riboflavin 50γ, pyridoxine HCl 30γ, calcium pantothenate 100γ, niacin 1 mg., inositol 1 mg., para-aminobenzoic acid 1 mg., choline 3 mg., concentrated extract of rice polishings (Galen B) 0.10 cc., vitamin A 133 I.U., vitamin D 18 I.U., alpha-tocopherol 1 mg.; Supplement B, experiments 3, 4, 5, and 6: thiamine HCl 20γ, riboflavin 20γ, pyridoxine HCl 20γ, calcium pantothenate 100γ, niacin 66γ, inositol 2.5 mg., para-aminobenzoic acid 100γ, choline 5 mg., folic acid 20γ, biotin 2γ, vitamin A 100 I.U., vitamin D 10 I.U., alpha-tocopherol 1 mg.

Exp. No.	*Experimental Period (weeks)*	*Preliminary Basal Diet*	*Vitamin Supplement*	*Nitrogen in Diets*[a]	*Method of Feeding*	*Diet*[b]
				%		
1	4	Protein-free	A	1.88	Equalized	Bread: A,B Nonfat dry milk F
2	5	Protein-free	A	1.88	Equalized	Bread: G,H,J,K,L
3	4	Protein-free	B	1.95	*Ad libitum*	Bread: M,O Bread ingred.: N,P
4	4	6% Casein	B	2.00	*Ad libitum*	Bread: R,T,U,W,Y Bread ingred.: S,V,X,Z
5	4	6% Casein	B	1.95	*Ad libitum*	Bread: AA,BB,DD Bread ingred.: CC,EE
6	4	6% Casein	B	1.88	*Ad libitum*	Bread: II,III,V,VI,VIII,IX Bread ingred.: I,IV,VII

[a] Percentage ± 0.02%.
[b] For explanation of diets, see Table I.

In experiments 1, 2, and 3, a protein-free basal diet was fed during this preliminary period. So that the effects of a protein-free diet would not be superimposed upon the shock of being weaned and isolated, the rats used in experiments 4, 5, and 6 were given a diet containing 6% casein instead of the basal diet used in earlier experiments. At the end of this preliminary period, the animals were weighed and arranged into groups as nearly alike as possible with respect to weight, sex, and litters represented. The rats in each group were fed and given vitamin supplements three times a week and were given water *ad libitum*. Weight gains and food consumption were recorded weekly. In experiments 1 and 2, the food intakes of the animals were kept as nearly equal as possible. A rat that consumed its food before the others had finished theirs was given a small amount of the protein-free basal diet. Since this practice results in a lowering of the percent protein ingested by the animals with the larger appetites, the rats used in experiments 3, 4, 5, and 6 were fed *ad libitum*.

The apparent digestibility of the dietary protein was determined in experiments 3, 4, and 5 by analyzing the feces for protein nitrogen and calculating the coefficients of digestibility from the ratios of the absorbed protein nitrogen to the protein nitrogen intake.

Method Used. The problem of finding the ideal method for the determination of protein quality has never been solved to the satisfaction of all. The functions of maintenance of the animal body, of repletion of protein stores in protein-deficient subjects, of growth, and of reproduction have all been utilized in determining nutritive values. In the present study the protein quality of the experimental breads was determined by the rat-growth method of Osborne, Mendel, and Ferry

(17), with certain modifications. Each bread was fed at one level of protein content rather than at several. It was assumed that the maximum protein efficiencies for all the breads would be similar enough to ensure that each protein efficiency determined would bear the same relationship to its maximum and that the results, though relative, would be comparable.

Boas Fixsen (7) and Mitchell (13) object to the method of Osborne *et al.* on the grounds that no allowance is made for the body maintenance of the animals, that the percentage protein in the body weight gains is assumed to be constant for animals on all experimental diets, and that *ad libitum* feeding produces large errors, thereby exaggerating large differences and obscuring small ones. Hegsted and Worcester, in 1947 (10), and Sherwood and Weldon, in 1953 (21), considered that the calculation of protein efficiency ratios was unnecessary and that the body weight gains of the animals correlated well enough with the protein efficiency ratios so that the additional labor involved in the calculations provided no advantage. Barnes *et al.*, in 1945 (4), Barnes and Bosshardt, in 1946 (3), and Barnes, Bates, and Maack, in 1946 (2), have developed modifications of Osborne, Mendel, and Ferry's method, designed to eliminate some of the objectionable features. As originally developed, the method requires that each protein source be fed at several levels in the diet in order to determine the maximum value of the protein efficiency ratios for each protein. As the protein content of the diet increases, the protein efficiency ratio increases to a maximum and then decreases in all except poorer-quality proteins, such as gluten. Since this method is cumbersome, many workers arbitrarily choose a single level of protein at which to feed the protein sources being tested. However, since proteins vary in the level in the diet at which the maximum protein efficiency occurs, some proteins will be seen at an advantage and some at an unfair disadvantage. Barnes *et al.* (4) have noted that the level of absorbed protein at which the maximal utilization for the synthesis of body protein occurs is similar for proteins of widely differing quality, wheat gluten, soy flour, and whole egg. The utilization of absorbed protein is defined as the gain in body protein per g. of dietary protein absorbed, and the maximum range occurs in young rats when 0.9 g. of protein, or from 0.75 to 1.0 g. per day, is absorbed. At the level in the diet at which this maximum utilization occurs, the protein efficiency ratio is at or near its maximum.

Mitchell's and Boas Fixsen's objections to this method because of its failure to allow for the protein used for maintenance of the body cannot be met. The animal's requirement for maintenance must be determined separately and cannot be determined directly in a *growing*

animal. Barnes, Bates, and Maack (2) have determined that the requirement for maintenance is the same in young and in adult animals, on the basis of body surface area. A growth experiment, however, must necessarily be a growth *and* maintenance experiment, since those functions cannot be separated.

In experiments 3, 4, and 5 the apparent digestibility of the bread proteins was determined and the amount of protein nitrogen absorbed was calculated. Half of the 18 diets tested fell within the prescribed range, the variation being from 0.64 to 1.19 g. of protein retained per day. Those for which the amount of absorbed protein was below 0.75 g. per day were: whole wheat–water breads M and R; whole wheat–water bread ingredients N; 6% nonfat dry milk–whole wheat breads O and BB, the latter baked at 500° F.; 6% nonfat dry milk–white bread Y; and 12% nonfat dry milk–whole wheat bread DD. Those for which the amount of absorbed protein was above 1 g. per day were: whole wheat–lysine bread ingredients V; and 6% nonfat dry milk–whole wheat bread ingredients X.

Barnes *et al.* (4) found that when the percent protein in a diet was increased beyond the level at which the maximum protein efficiency ratio was obtained, the protein efficiency decreased except in the case of the poorest-quality protein tested, wheat gluten, in which the protein efficiency ratio maintained a plateau maximum. Thus, an amount of protein absorbed at the upper limits of the range 0.75 to 1.0 g. per day is probably preferable to a lower amount, and a level of protein nitrogen in the diet between 1.95 and 2.00%, used in the experiments reported here, seems reasonable.

Results and Discussion

The results of the experiments are shown in Table I. Protein quality is expressed as g. gain in body weight per g. of protein eaten (protein efficiency ratio) as well as g. gain in body weight per g. of protein nitrogen eaten (and hence could be called protein nitrogen efficiency ratio). Because the factors used to convert wheat nitrogen and milk nitrogen to protein are quite different (5.7 for wheat endosperm, 5.83 for whole wheat, and 6.38 for milk), the factors used in the calculation of the protein efficiency ratios were computed on the basis of the percentage of wheat and of milk protein in the diets.

Effect of Nonfat Dry Milk. The experiments were designed to compare the growth of animals fed: whole-wheat bread containing no milk; 3% nonfat dry milk (an amount commonly used commercially in making white bread and the maximum amount used locally in making whole-wheat bread); 6% nonfat dry milk (the amount present in fresh,

TABLE I

INFLUENCE OF BAKING, OF LYSINE, AND OF NONFAT DRY MILK ON THE QUALITY OF PROTEINS IN WHEAT BREAD[a]

Exp. No.	Diet No.	No. of Rats	Gains in Body Weight		Total Food Intake	Total Nitrogen Intake	Factor for Converting Nitrogen to Protein	Total Protein Intake	Protein Nitrogen Efficiency[b]		Protein Efficiency[c]		
									Ratio	St. Dev.	Ratio	St. Dev.	Increase
			g.	%	g.	g.		g.					%
			Whole-wheat bread, 0% nonfat dry milk (NDM)										
1	A	8	21.4		168	3.21	5.83	18.7	6.65	±0.70	1.14	±0.12	
2[d]	G	9	29.6		233	4.37	5.83	25.5	6.77	±0.53	1.16	±0.09	
3	M	10	23.6		191	3.75	5.83	21.9	6.26	±0.77	1.07	±0.13	
4	R	9	28.2		200	3.98	5.83	23.2	7.05	±0.56	1.21	±0.10	
6	II[e]	10	30.0		237	4.48	5.83	26.1	7.19	±2.37	1.23	±0.41	
6	III	10	32.1		237	4.50	5.83	26.2	7.17	±0.66	1.23	±0.11	
		Av.	*27.5*						*6.85*		*1.17*		
			White bread, 6% NDM										
2[d]	L	9	30.7		230	4.28	5.80	24.8	7.18	±0.64	1.24	±0.11	
4	Y	9	33.3		203	4.16	5.80	24.1	8.27	±1.02	1.43	±0.18	
6	VIII[e]	10	28.1		219	4.12	5.82	24.0	6.75	±1.02	1.16	±0.18	
6	IX	10	29.7		218	4.05	5.82	23.6	7.35	±0.79	1.26	±0.14	
		Av.	*30.4*	11					*7.39*		*1.27*		8
			Whole-wheat bread, 3% NDM										
1	B	9	27.9		179	3.32	5.88	19.5	8.39	±0.46	1.43	±0.08	
2[d]	H	9	38.3		263	4.94	5.88	29.0	7.78	±0.68	1.32	±0.12	
		Av.	*38.1*	38					*8.08*		*1.38*		18
			Whole-wheat bread, 6% NDM										
2[d]	J	9	45.2		260	4.88	5.91	28.8	9.26	±0.74	1.57	±0.13	
3	O	10	35.3		211	4.11	5.91	24.3	8.57	±0.50	1.45	±0.08	
4	W	9	48.3		253	5.45	5.90	32.1	9.50	±0.85	1.61	±0.14	
5	AA	10	44.5		233	4.58	5.90	27.0	9.87	±0.95	1.67	±0.16	
5	BB[f]	10	40.9		232	4.51	5.90	26.6	9.05	±0.52	1.53	±0.09	
		Av.	*43.3*	58					*9.30*		*1.58*		35

TABLE I (Cont'd)

Exp. No.	Diet No.	No. of Rats	Gains in Body Weight		Total Food Intake	Total Nitrogen Intake	Factor for Converting Nitrogen to Protein	Total Protein Intake	Protein Nitrogen Efficiency [b]		Protein Efficiency [c]		
									Ratio	St. Dev.	Ratio	St. Dev.	Increase
			g.	%	g.	g.		g.					%
			Whole-wheat bread, containing 0.166% l-lysine										
4	T[e]	9	48.1		247	5.14	5.83	30.0	9.75	±0.63	1.67	±0.11	
4	U[e]	9	49.3		251	5.08	5.83	29.6	9.46	±1.01	1.62	±0.17	
		Av.	*49.2*	42					*9.60*		*1.65*		40
			Whole-wheat bread, 12% NDM										
2[d]	K	9	49.1		273	5.13	5.96	30.6	9.58	±1.04	1.61	±0.17	
5	DD	10	39.7		230	4.42	5.95	26.3	8.95	±0.67	1.50	±0.11	
6	V[e]	10	58.7		300	5.61	5.95	33.4	10.45	±1.19	1.76	±0.20	
6	VI	10	56.1		302	5.73	5.95	34.1	9.73	±1.06	1.64	±0.18	
		Av.	*50.9*	85					*9.68*		*1.63*		39
			Unbaked ingredients: Whole-wheat flour, 0% NDM										
3	N	10	34.4		207	4.00	5.83	23.3	8.58	±0.78	1.47	±0.13	
4	S	9	42.3		241	5.14	5.83	30.0	8.67	±0.92	1.49	±0.16	
6	I	10	45.0		264	5.00	5.83	29.2	8.72	±0.96	1.50	±0.16	
		Av.	*40.6*	..					*8.66*		*1.49*		..
			Unbaked ingredients: White flour, 6% NDM										
4	Z	9	47.2		254	5.32	5.80	30.9	9.33	±0.83	1.61	±0.14	
6	VII	10	50.5		279	5.28	5.82	30.7	9.57	±0.91	1.64	±0.16	
		Av.	*48.8*	20					*9.45*		*1.62*		9
			Unbaked ingredients: Whole-wheat flour, 6% NDM										
3	P	10	45.4		235	4.54	5.91	26.8	9.99	±1.06	1.69	±0.18	
4	X	9	72.8		306	6.23	5.90	36.8	11.81	±0.70	2.00	±0.12	
5	CC	10	69.2		273	5.74	5.90	33.9	12.10	±0.83	2.05	±0.14	
		Av.	*62.4*	54					*11.30*		*1.91*		28

TABLE 1 (Completed)

Exp. No.	Diet No.	No. of Rats	Gains in Body Weight		Total Food Intake	Total Nitrogen Intake	Factor for Converting Nitrogen to Protein	Total Protein Intake	Protein Nitrogen Efficiency [b]		Protein Efficiency [c]		
									Ratio	St. Dev.	Ratio	St. Dev.	Increase
			g.	%	g.	g.		g.					%
			Unbaked ingredients: Whole-wheat flour, 0.166% l-lysine										
4	V	9	73.7	82	300	6.35	5.83	37.0	11.58	±1.12	1.99	±0.19	34
			Unbaked ingredients: Whole-wheat flour, 12% NDM										
5	EE	10	75.7		280	5.57	5.95	33.1	13.58	±0.86	2.28	±0.14	
6	IV	9	87.7		353	6.67	5.95	39.7	13.17	±0.75	2.21	±0.13	
		Av.	*81.7*	101					*13.38*		*2.24*		50
			Unbaked ingredient: Nonfat dry milk										
1	F	9	56.1	37	190	3.69	6.38	23.5	15.22	±0.99	2.39	±0.16	60

[a] Experimental period, 4 weeks. The diets in experiments 1, 2, and 6 contained 1.88 ± 0.02% nitrogen; 3 and 5, 1.95 ± 0.02% nitrogen; 4, 2.00 ± 0.02% nitrogen.
[b] Expressed as gains in body weight per g. of nitrogen intake.
[c] Expressed as gains in body weight per g. of protein intake.
[d] Experimental period 5 weeks.
[e] Dried at room temperature.
[f] Baked at 500°F. Data not included in averages.
[g] Added to diet.

fluid nonfat milk sufficient to prepare the dough without use of other liquids); 12% nonfat dry milk; and white bread containing 6% nonfat dry milk.

From Table I it can be seen that, as the amount of nonfat dry milk in whole-wheat bread increased, the gains in body weight of the animals increased. Animals receiving the whole wheat-water bread containing 6% nonfat dry milk had greater gains than those receiving the white bread containing 6% milk.

Data on the effect of nonfat dry milk on the protein quality of breads and of the unbaked ingredients are also given in Table I. To test the significance of the differences between similar protein nitrogen efficiency ratios, t values were calculated. For ratios differing widely, no statistical test was deemed necessary. From this table it can be seen that the addition of nonfat dry milk to whole-wheat bread at the levels of 3 and 6% effectively improved the protein quality. The addition of 12% milk caused either a slight but not statistically significant improvement (experiment 2), or a decrease in degree of improvement (experiment 5) as compared with the 6% milk. The addition of 6% nonfat dry milk to unbaked whole-wheat bread ingredients resulted in a significant improvement in protein quality over that of the ingredients without milk, and the addition of 12% nonfat dry milk caused a significant improvement over that of 6% milk.

The nutritive value of the proteins of white bread made with 6% nonfat dry milk, which was included for the purpose of comparison with the whole-wheat breads, ranked superior to whole-wheat bread made without milk, slightly but not significantly inferior to whole-wheat bread made with 3% nonfat dry milk, and significantly inferior to whole-wheat bread containing 6% nonfat dry milk. White bread ingredients containing 6% nonfat dry milk were slightly but not significantly superior to unbaked whole-wheat bread ingredients with no milk, and significantly inferior to whole-wheat bread ingredients with 6% milk.

The sample of nonfat dry milk used in experiment 1 was of somewhat inferior baking quality, slightly dark in color, and had an off-flavor – the latter two characteristics probably being due to poor storage conditions. The nutritive quality of the protein, however, was only very slightly impaired. The protein efficiency ratio was 2.40, as compared with 2.45 obtained by Cook *et al.* in 1951 (9) in the same laboratories, with a sample which had been prepared for baking use and properly stored. The milk samples used in experiments 2, 3, 4, 5, and 6 were of good baking quality with no off-odor or color.

It is recognized that the determination of the apparent digesti-

bilities of the breads showed that the white bread and unbaked white bread ingredients had a higher average digestibility (88.2%) than did the whole-wheat breads and unbaked whole-wheat bread ingredients (83.1%), and that, were the evaluation of protein quality based upon the weight gains per g. of protein absorbed rather than upon the weight gains per g. of protein eaten, the quality of the whole-wheat breads would appear to be enhanced.

The addition of milk to whole-wheat breads (baked and unbaked) had little effect on the apparent digestibility. Whole-wheat bread fed to four groups of animals showed an average digestibility of 83.3%; whole-wheat bread made with 6% nonfat dry milk fed to eight groups showed an average digestibility of 84.0%; and whole-wheat bread made with 12% dry milk fed to two groups showed an average digestibility of 81.1%.

Effect of Fermentation and Baking upon the Protein of Breads. None of the many previous studies upon the protein quality of bread and of wheat and wheat flour has compared the nutritive value of the bread and of the unbaked ingredients from which the bread was made. The moderate temperatures achieved in boiling cereals have been reported to have no deleterious effect on their protein quality (5, 14, 16). Stout and Drosten, in 1933 (22), studying time-temperature relationships in the baking of bread, found that the interior temperature of loaves of bread did not exceed 100° to 102°C. during the baking procedure, such temperatures being held for 9–12 minutes to achieve doneness. As shown by Barackman and Bell in 1938 (1), however, the temperature of the crust rises rapidly to 100°C., and then to as high as 150°C. as the dehydration of the dough progresses and the crust forms and caramelizes to a desirable degree of brownness. Studies in 1931 by Morgan (16) and by Kon and Marcuze (12) have shown that, while the protein quality of the crust of bread is lower than that of the crumb, the protein quality of the crumb and of the whole loaf is the same. In view of these findings, it might be assumed that the process of baking would not lower the protein quality of the bread ingredients.

The investigation here reported, however, has shown that the protein nitrogen efficiency ratios of whole-wheat bread containing 0, 6, and 12% nonfat dry milk and of white breads made with 6% nonfat dry milk were an average of about 20% lower than those of the corresponding unbaked bread ingredients, as can be seen from Tables I and II. The differences are highly significant for all breads as tested by calculation of the *t* values. Two exceptions occurred in one group of animals fed whole-wheat bread with no milk and in one group fed

white bread with 6% nonfat dry milk; in both of these cases the differences were significant at the 5% level.

TABLE II

EFFECT OF FERMENTATION AND BAKING ON THE PROTEIN QUALITY OF BREADS

Experiment No.	Diet	Protein Nitrogen Efficiency Ratio and Standard Deviation		Decrease in Protein Nitrogen Efficiency Ratio on Baking		t Value
		Ingredients	Bread			
					%	
3	Whole-wheat bread, no milk: N and M	8.58±0.78	6.25±0.77	2.32	27	6.90 **
4	S and R	8.67±0.92	7.05±0.56	1.62	19	4.83 **
6	I and III	8.72±0.96	7.17±0.66	1.55	18	4.08 **
6	I and II	8.72±0.96	7.19±2.37	1.63	19	1.88 *
3	Whole-wheat bread, 6% NDM[a]: P and O	9.99±1.06	8.57±0.50	1.42	14	3.90 **
4	X and W	11.81±0.70	9.50±0.85	2.31	20	4.42 **
5	CC and AA	12.10±0.83	9.87±0.95	2.23	18	5.58 **
5	Whole-wheat bread, 12% NDM: EE and DD	13.58±0.86	8.95±0.67	4.63	33	13.87 **
6	IV and VI	13.17±0.75	9.73±1.06	3.44	26	10.86 **
6	IV and V	13.17±0.75	10.45±1.19	2.72	21	6.03 **
4	White bread, 6% NDM: Z and Y	9.33±0.83	8.26±1.02	1.07	11	2.44 *
6	VII and IX	9.57±0.91	7.35±0.79	2.22	23	5.82 **
6	VII and VIII	9.57±0.91	6.75±1.02	2.82	29	6.85 **
				av.	21	

[a] Nonfat dry milk.

An examination of data concerning preparation of diets fed the experimental animals suggested that the method of drying bread to standardize the diets might explain some of the decrease in protein quality with baking. The baked breads were sliced thin and dried in a home dehydrator set at the lowest temperature possible. The maximum heat attained within the cabinet was 50°C. (122°F.). Block *et al.* (6) noted a decrease in protein efficiency ratio of a high-protein rusk dried at 60°C. as compared with the protein efficiency ratio of the same rusk air-dried at room temperature. Cook *et al.* in 1951 (8) reported that an initial heating of casein or lactalbumin with lactose at 52°C. for 24 hours resulted in slight heat damage with respect to availability to the rat, but that subsequent heating of the heat-treated proteins caused a marked decrease in protein quality. It is therefore likely that not all the heat damage to the proteins of the various breads tested occurred in the baking, but that at least a part of the injury occurred in the drying of the breads after baking.

TABLE III

COMPARISON OF PROTEIN NITROGEN EFFICIENCY RATIOS FROM BREADS DRIED AT ROOM TEMPERATURE AND DRIED IN A DEHYDRATOR AT TEMPERATURES UP TO 50° C.

Diet	Type of Bread in Ration	Drying Treatment	Protein Nitrogen Efficiency[a]			t Value
			Ratio	St. Dev.	Difference	
II	Whole-wheat, no milk	Room temp.	7.19	±2.37	−0.02	0.03
III	Whole-wheat, no milk	Dehydrator	7.17	±0.66		
V	Whole-wheat, 12% NDM[b]	Room temp.	10.45	±1.19	−0.72	1.42
VI	Whole-wheat, 12% NDM	Dehydrator	9.73	±1.06		
VIII	White, 6% NDM	Room temp.	6.75	±1.02	+0.60	1.23
IX	White, 6% NDM	Dehydrator	7.35	±0.79		

[a] Gain in body weight per gram of protein nitrogen intake.
[b] Nonfat dry milk.

Consequently, an experiment was designed to compare whole-wheat breads containing no milk and 12% nonfat dry milk, and white bread with 6% nonfat dry milk, dried at room temperature, with the same breads dried in the dehydrator under conditions that had prevailed in previous experiments. The results (Tables I and III) showed that there was no significant difference between the whole wheat water breads dried at room temperature and those dried in the dehydrator. The dehydrated bread containing 12% nonfat dry milk had a slightly lower protein nitrogen efficiency ratio than did the bread dried at room temperature, while the dehydrated white bread containing 6% nonfat dry milk had a slightly higher protein nitrogen efficiency ratio than did the corresponding bread dried at room temperature. Neither of these differences was statistically significant, however, and it can be concluded that drying at temperatures up to 50°C., rather than drying the bread at room temperature, did not in itself account for the large differences in protein quality found between the baked breads and the unbaked ingredients.

Supplementation of Whole Wheat and Whole-Wheat Bread with Lysine. Investigations on the deficiency of wheat proteins in the amino acid lysine have been made by many workers and include studies on gliadin and white flour, with rats as subjects; gluten, using human subjects, dogs, and chicks; whole wheat fed to rats and chicks; and white bread, using rats and human subjects (18, 19, 20). In none of this research was the supplementary effect of amino acids on the protein quality of whole-wheat bread investigated, and the only research on whole wheat was that of Mitchell and Smuts (15), using a supplement of 0.25% l-lysine, and of Sure (23), using a supplement of 0.2% l-lysine, both experiments with rats.

In order to compare the supplementary effect of milk proteins and

lysine on the proteins of whole-wheat bread and bread ingredients, 0.166% l-lysine (the amount present in 6% nonfat dry milk) was included, as 0.207 g. l(+) lysine monohydrochloride, in the formula for water whole-wheat bread. The sample of heat-treated spray-dried nonfat dry milk analyzed 2.77% lysine by microbiological assay from an acid hydrolysate of the milk. The assay was effected by the use of *Leuconostoc mesenteroides* P-60 with an incubation period of 72 hours at 37°C. A complete basal medium was used.[3] The crust color of the bread containing lysine was darker than that of either the water bread or the bread containing 6% nonfat dry milk. Rosenberg and Rohdenburgh (18) noted that their white bread containing lysine had a darker crust than did their control bread. They found no difference in texture. In the present experiment, the bread made with lysine was more compact than the bread made with water or milk.

TABLE IV

EFFECT OF LYSINE ON THE PROTEIN QUALITY OF WHOLE-WHEAT BREAD

Type of Ration	Diet	Supplement	Protein Nitrogen Efficiency				
			Ratio	St. Dev.	t Values		Increase
							%
Whole-wheat bread	R	.....	7.05	±0.56			
	T	0.166% l-lysine	9.75	±0.63	6.28**	compared with R	38
	W	6% nonfat dry milk	9.50	±0.85	0.49	compared with T	35
Whole-wheat ingredients	S	.....	8.69	±0.92			
	V	0.166% l-lysine	11.58	±1.05	6.22**	compared with S	33
	X	6% nonfat dry milk	11.81	±0.70	0.29	compared with V	36
Whole-wheat bread	T	0.166% l-lysine added before baking	9.75	±0.63			
	U	0.166% l-lysine added after baking	9.46	±1.01	0.53		

The results of the experiment on the effect of lysine on the protein quality of whole-wheat bread are given in Tables I and IV. The gain per g. of protein in rats fed the lysine bread was the same as that in rats fed whole-wheat bread made with 6% nonfat dry milk, and greater than that of rats fed water whole-wheat bread. Similar results were observed when rats were fed the corresponding unbaked ingredients. From this evidence, milk added at the 6% level contributes chiefly lysine to supplement the proteins of wheat. At higher levels, as the lysine deficiency of wheat is filled, the milk may supply other amino acids to make up the less acute deficiencies of the wheat proteins. The

[3] H. M. Chemical Company, Ltd., Los Angeles, Calif.

baking and drying losses of the lysine whole-wheat bread were 16% and were similar to those of the milk breads (see Table II). Before attributing the entire supplementary effect of milk proteins on wheat proteins to the lysine content of the milk, it should be noted that the proteins of the bread baked with lysine contained a higher percent lysine than did the proteins of the bread baked with nonfat dry milk, the remainder of the amino acids of the added milk proteins in the latter bread serving to dilute the lysine content. The dilution of the added lysine with nonessential amino acids to make up an amount equivalent to the protein content of 6% nonfat dry milk would have given a more accurate picture of the contribution of the lysine in that amount of milk.

In the only comparable work, that of Sure in 1952 (23), the protein was fed at a level of 8%, as contrasted with a level of 11.5% in this experiment. The results for comparable groups showed increases of the same magnitude due to the addition of lysine – an increase in protein efficiency ratio from 1.22 to 1.77 caused by adding 0.2% lysine to whole wheat – while the experiment reported here showed an increase from 1.49 to 1.99 caused by adding 0.166% lysine to unbaked whole-wheat bread ingredients.

In order to determine how much of the added lysine was made unavailable to the rats by the baking process, the protein efficiency ratio of the bread made with 0.166% lysine was compared with that of bread made without lysine to which 0.166% lysine was added after the bread was baked, dried, and ground. The results show no difference in protein nitrogen efficiency ratio between the two diets (Table IV). This finding agrees with that of Rosenberg and Rohdenburgh (18, 19), who found that, when 0.2% lysine was added before and after baking, either as dl-lysine hydrochloride or l-lysine hydrochloride, a loss of 15% l-lysine occurred with the former and a loss of 32% with the latter, but the protein efficiency ratios of the two breads when fed to rats were the same.

Effect of Variation of Baking Time and Temperature on the Protein Quality of Whole-Wheat Bread. Commercial bakers often increase their output of bread during a given period of time by increasing the oven temperature and decreasing the baking time. One local plant bakes bread for 25 minutes at 500°F. The protein quality of a whole-wheat bread made with 6% nonfat dry milk baked under these conditions was compared with that of bread made from the same formula baked 35 minutes at 430°F. The crusts of the two types of loaves seemed to be of the same brownness and the interiors of both seemed to be well baked.

When these breads were fed to rats, the bread baked at the higher temperature for the shorter time gave a protein nitrogen efficiency ratio of 9.05 ± 0.52 as compared with 9.87 ± 0.95 for the bread baked at the lower temperature for the longer time. The difference here is slightly significant ($t = 2.37$, a t value of 2.09 being significant at the 2.5% level, and higher values being more significant). It would be necessary to feed a greater number of animals these two breads to certify that the bread baked at the lower temperature for the longer time is superior nutritionally to the bread baked at the higher temperature for the shorter time.

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THE RATES OF PENETRATION OF MOISTURE TO DIFFERENT POINTS IN THE CENTRAL CROSS-SECTION OF THE ENDOSPERM IN DAMPED MANITOBA WHEAT GRAINS[1]

J. D. CAMPBELL AND C. R. JONES

ABSTRACT

In damped Manitoba wheat grains kept at 20°C., about one-third of the final moisture increment is relatively rapidly reached in all parts of the endosperm, but the rate of penetration in the later stages of moisture distribution varies in different parts of the central cross-section. The pattern of the variation follows that of the demarcation of the types of endosperm cell, penetration being more rapid in the prismatic than in the rounded-cell region. The endosperm near the crease and in the cheek center is last to reach the final level of moisture content.

The rate of movement of moisture into the cheek centers of damped Manitoba wheat grains kept at 20°C. (68°F.) has been studied by means of microdetermination of the density of dissected endosperm particles (4) and, subsequently, the effect of elevation of temperature during the period of storage of the damped grain was followed by similar means (1). The present paper gives a comparison of the rates of penetration to different points in the cross-section of the endosperm in damped Manitoba grains which have been kept at 20°C. after damping.

[1] Manuscript received July 2, 1956. Contribution from the Research Association of British Flour Millers, St. Albans, England.

Materials and Methods

Wheat. The sample of No. 1 Manitoba Northern wheat, described previously (4) as "Manitoba A," was used.

Moisture contents were determined by the method already given (4). All moisture contents are expressed on the moist basis. In order to ensure uniform damping, the wheat (at 12.6% moisture content) was graded in grain size and wetted by immersion for 1 minute, followed by centrifuging, the aim, as previously described (1), being to obtain grains at 17.2% moisture content. The damped wheat was stored in small air-tight jars in a constant-temperature chamber at 20°C. In the particular case (Table II) where the damping was carried only to 14.8% moisture content, the grains were rolled gently between layers of damped cloth until the desired extent of moisture pick-up had been obtained.

Densities of particles dissected (under oil) from different positions in the endosperm were determined by the method, depending on suspension of the particles in water-saturated mixtures of carbon tetrachloride and cyclohexane, previously given (4). As before, values for density are expressed on the $^{25°}/_{25°}$ basis.

Interpretation of Moisture Contents from Density Values. In the case of particles from the cheek centers, density values may be converted into values for percentage moisture content by means of the graph previously given (4, Fig. 3). This graph showed a linear relationship between density and moisture content over the useful range, the gradient being 0.0041 per 1% difference in moisture content. It applied equally well to particles from the cheek centers of vitreous grains of many different wheats. The present study concerns endosperm particles from six different positions (additional to the cheek center) in the central cross-section, as shown approximately to scale in Fig. 1. The variation in density of the endosperm from point to point in a central cross-section of vitreous Manitoba grain at 12.6% moisture content was shown in a previous diagram (4, Fig. 2); the points shown included the present six positions. The differences in density between endosperm particles from these positions in grain at 12.6% moisture content are now shown in Fig. 1 in the form of single numerals, each of which represents the value in the third decimal place by which the density at the respective point falls short of that in the cheek center.

Further data, given in Table I, show that these differences are maintained at raised moisture contents. In other words, lines drawn to relate density with moisture content would be closely parallel for all these positions; in the case of the outer dorsal endosperm this was also

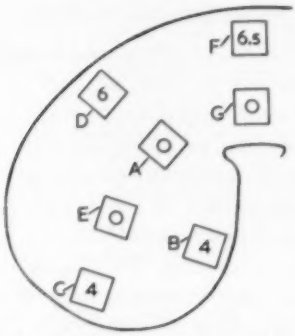

Fig. 1. Allowances to be added to density values before converting them into moisture contents by means of the graph (*ref.* 4, Fig. 3) relating density and moisture content of endosperm from the central cheek region in vitreous grains. The allowances are here shown as values, in the third decimal place, by which the density at the respective points in the central cross-section of the grain falls short of that in the cheek center.

established by data (not given here) for a series of moisture contents on the lines of those from which the graph (4, Fig. 3) for the cheek center was drawn.

Accordingly, a density value for endosperm in any one of the positions studied may be converted into a value for moisture content by reading from the central-cheek graph (4, Fig. 3), provided that the appropriate allowance for position, read from the present Fig. 1, is first added to the experimentally determined density value.

Results

Mean values for density were determined on particles of endosperm dissected from each of three representative vitreous grains taken from damped Manitoba wheat after various periods of storage at 20°C. These values were converted into moisture contents as described under "Materials and Methods." Figure 2 shows these moisture contents

TABLE I

DENSITIES, AT MOISTURE CONTENTS 12.6% AND 17.2%, OF ENDOSPERM FROM DIFFERENT REGIONS IN THE CENTRAL CROSS-SECTION OF VITREOUS MANITOBA GRAINS

LOCATION		DENSITY		
Symbol[a]	Description	12.6% Moisture[b]	17.2% Moisture	Difference
E	Cheek, center	1.4580	1.4394	0.0186
A	Shoulder, center	1.4580	1.4395	0.0185
D	Shoulder, outer	1.4520	1.4329	0.0191
C	Cheek periphery, ventral	1.4540	1.4353	0.0187
B	Cheek periphery, crease	1.4540	1.4352	0.0188
F	Dorsal periphery, outer	1.4515	1.4322	0.0193
G	Dorsal periphery, crease	1.4580	1.4391	0.0189

[a] The symbols relate to Fig. 1.
[b] From literature reference 4, Fig. 2.

plotted against period of storage of the damped grain. The particles were dissected from the seven positions in the cross-section of each grain represented by *A* to *G* in Fig. 1. Of these, *E* corresponds to the cheek center previously studied (4, Fig. 2). *A* is also at a point midway through the endosperm but in the shoulder of the grain, in part of the prismatic endosperm cell region. The remaining five points lie nearer to the periphery of the endosperm, at representative positions in a direction around the grain.

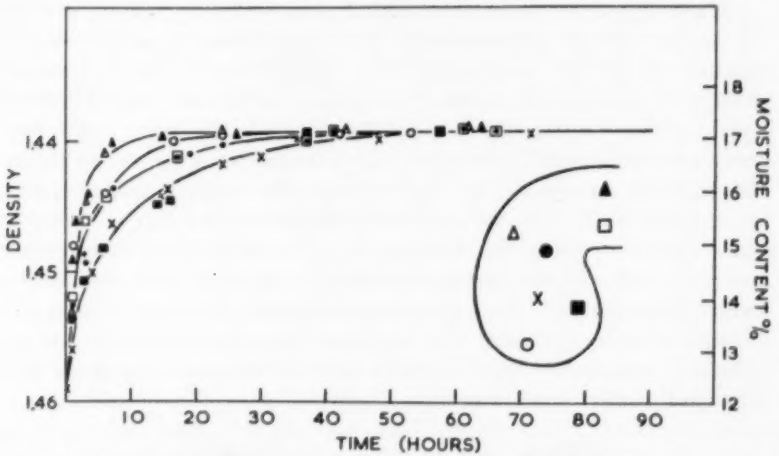

Fig. 2. Rate of penetration of moisture at 20°C. to various points in the central cross-section of damped Manitoba wheat as shown by the density of the endosperm at various times after damping. The experimentally determined density values have been adjusted by means of the appropriate allowances for position shown in Fig. 1 before being plotted here.

It is evident from Fig. 2 that the peripheral endosperm around the back and shoulders of the grain absorbs moisture from the damped bran considerably more rapidly than that around the cheeks. Thus, from Fig. 2, the periods required for endosperm in different positions in the cross-section of the damped grain to rise in moisture content to 16% and 17% respectively are:

	Endosperm near periphery			
Moisture content	Dorsal, F[a]	Shoulder, D[a]	Ventral, C[a]	*Central cheek*
%	*hrs.*	*hrs.*	*hrs.*	*hrs.*
16	2½	2½	4–5	13
17	10	10	16–17	48

[a] These symbols refer to Fig. 1.

Figure 2 shows, further, that the penetration to the endosperm near the periphery of the cheek within the crease is as slow as that to the cheek center.

Previous observations by means of the iodine vapor staining method (3), on decorticated grains immersed in water, indicated that the rate of penetration through the endosperm was greatest near the back of the grain and that it decreased towards the face of the cheek; there was no entry near the crease. Hinton (2) also found, by measuring the rate of movement of water from a capillary tube attached to the endosperm, that the permeability of the endosperm on the back of Thatcher grain was somewhat greater than that in the cheek.

These previous observations related to phenomena possibly involving mechanisms of a different kind from those operating in the present series of experiments. In the previous cases contact with excess of liquid water was maintained; in the present case, a limited proportion of moisture was applied initially to the exterior of the grain and subsequent movement within the grain may have been via the vapor phase. Nevertheless, the parallelism regarding relative penetrabilities of different parts of the endosperm is such that the two series of observations support one another. Incidentally it is not possible from either series to attribute the low penetrability, apparent near the crease, merely to mechanical limitation of initial access of water to the bran situated within the crease. The results simply show that, in grain as ordinarily moistened, the endosperm near the crease is among the last to reach the final level of moisture content.

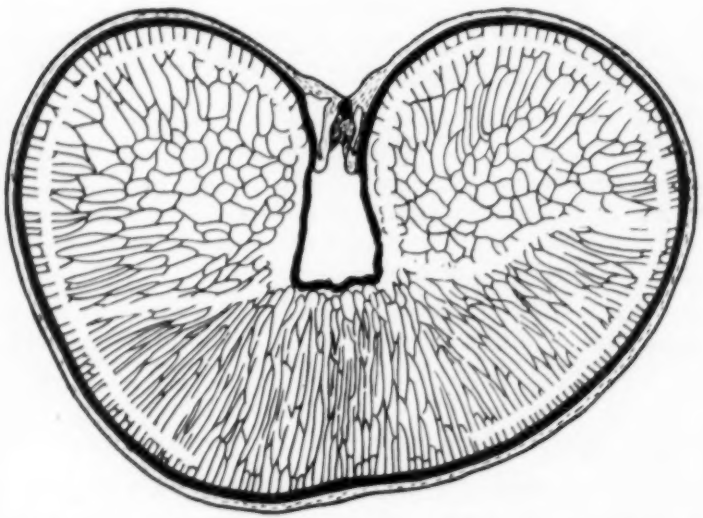

Fig. 3. Transverse section of Manitoba wheat grain (from ref. 5, Fig. 1). Areas occupied by peripheral, prismatic, and central types of endosperm cells are delineated by white bands breaking the continuity of the cell-wall outlines.

It is also evident from Fig. 2 that rates of penetration to the innermost endosperm situated at different points in the dorsal region are much alike, and that they are greater than that to the cheek center. If it is assumed that the bran situated within the crease is not accessible to water during the immersion period, Fig. 2 affords in effect a comparison of rates of penetration to points situated in an arc distant from the grain periphery by half the cheek width (i.e., almost the whole depth of the dorsal part of the grain). Reference to Fig. 3, which is a diagram (reproduced from ref. 5, Fig. 1) of endosperm cell structure within the grain, then suggests that these rates are similar so long as the points lie in or near the prismatic endosperm cell region, but are markedly lower when the points lie in the region occupied by the central-type or rounded endosperm cells. It has previously been suggested (6) that moisture entering the grain may follow the endosperm cell walls, and even that it may not necessarily penetrate within the actual cells. No experimental evidence was given, however, nor were differences between various parts of the grain mentioned. The connection between the present results and endosperm cell configuration is no more than circumstantial but it is not unnatural to suppose that the interstices between the prismatic cells may conduct moisture towards the grain interior more readily than those between the rounded cells, since, in contrast to the latter, they are straight, disposed radially with respect to the long axis of the grain, and relatively numerous owing to the narrowness of the cells. It is also relevant that other evidence from these laboratories (5) showed that the endosperm in the region of the rounded cells is particularly resistant to mellowing during tempering of wheat (cold-conditioning) in preparation for milling.

The above discussion, however, relates essentially to the later stages of moisture distribution. On the other hand, Fig. 2 also shows the possibly surprising rapidity with which a minor portion of the added moisture penetrates to all parts of the endosperm. All regions, including even the cheek centers, have received one-third of their eventual increment in just over 2 hours. The further step, between one-third and one-half, marks the commencement of differentiation, between the cheek and the remainder of the endosperm, in rate of receipt of moisture.

The amount of moisture involved in this exceptionally rapid migration is not absolute but is related to the extent of initial damping of the grain, i.e., to the level of water content to which the outer layers of the grain have been brought. This is apparent from Table II, giving comparative data on Manitoba grains which were moistened initially,

TABLE II

CHANGES IN ENDOSPERM OF MANITOBA WHEAT AT CHEEK CENTER IN 2½ HOURS AT 20°C. AFTER MOISTENING TO DIFFERENT EXTENTS

STATE OF CHEEK CENTER AFTER 2½ HOURS	FINAL MOISTURE CONTENT OF GRAIN	
	14.8%	17.2%
Density	1.4545	1.4515
Moisture content (%)	13.4	14.1
Moisture increment as proportion of total increment in grain (%)	34.6	31.5

on the one hand to 17.2% (as in the cases previously discussed in this paper) and on the other hand only to 14.8%, and then kept at 20°C. The table shows values for density of particles dissected from the central cheek region of each lot of grain only 2½ hours after the grain had been damped. It also shows the corresponding indicated moisture contents at the cheek centers and the increments shown by these values, expressed as percentages of the total moisture increment of the grain in each case (the percentages being based on the weight of the grain before damping).

The results show that both with the lightly and the more heavily moistened grain the particularly rapid initial rise in moisture content of the endosperm at the cheek center was limited to about one-third of the eventual equilibrium value.

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A SHORT METHOD FOR STRUCTURAL RELAXATION IN DOUGH[1]

I. HLYNKA

ABSTRACT

Structural relaxation parameters describing dough properties are obtained from duplicate extensograms at rest periods of 10 and 75 minutes by simplified procedure. The correlation coefficients for the relation between the data by the short method and the regular procedure were 0.96 for the relaxation constant and 0.98 for the asymptotic load. It is suggested that for routine testing the dough properties be characterized initially at a 5 minute reaction time; and then at a reaction time of 3 hours, once without bromate and again with 20 p.p.m. bromate.

A method of describing dough properties by means of a structural relaxation curve obtained from extensogram measurements has been described in several publications from this laboratory (4, 5, 6). Very briefly, the method consists of the following. A series of extensograms (usually six) is obtained on samples of dough differing only in the time intervals or rest periods (usually 5 to 105 minutes) allowed between shaping and stretching the test samples. The extensogram load read at constant sample deformation (usually 7 cm. on the extensogram) is then plotted against the rest period to give the structural relaxation curve. Finally, the relaxation curve, which may be assumed to be a hyperbola, is transformed mathematically into a linear form, and two constants describing the curve, and hence the dough properties, are evaluated by the method of least squares.

The structural relaxation method introduced two main features. First, the primary extensogram measurement adopted is the load supported by the dough, not at the curve maximum but at a constant extension of the test piece. Loads (or resistances) for different doughs are thus compared under conditions of essentially the same dough sample dimensions, analogous to a measurement of stress. Second, the mathematical constants describing the dough properties are calculated over a range of rest periods and represent the dynamic behavior of the dough. In contrast, the dimensions of any single extensogram represent the properties of the dough only at the specific time at which the extensogram is drawn; i.e., they depend on the particular rest period between shaping the test sample and stretching it. For these reasons the structural relaxation method is considered to provide a

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more comprehensive and hence a more meaningful measurement of dough properties.

Although the new technique of structural relaxation has been useful in a variety of fundamental studies on the chemistry and rheology of dough (1–6), the method in its present form is too complex for routine use in control laboratories. Accordingly, a simplified structural relaxation procedure has been developed for routine control and is described in this paper. The mathematical basis of the short method is presented first, and this is followed by illustrative data.

Mathematical Basis of the Short Method for Structural Relaxation

It has been pointed out in a previous publication (4) that the structural relaxation curve can be described to a good approximation by the equation of a hyperbola of the form

$$(L - L_A)t - C = 0 \tag{1}$$

where L is the extensogram load at constant sample extension at rest period t, L_A is the theoretical load which the relaxation curve approaches asymptotically, and C is a constant related to the curvature of the hyperbola. This equation can easily be rearranged into an equation of a straight line

$$(Lt) = L_A t + C \tag{2}$$

where the dependent variable is (Lt), the product of load and rest period, the slope is L_A, and the intercept is C.

To evaluate the constants L_A and C, a minimum of two experimental points is required. If L_1 and L_2 are the loads at rest periods t_1 and t_2, then by standard procedures

$$L_A' = \frac{(L_2t_2) - (L_1t_1)}{t_2 - t_1} \tag{3}$$

$$\text{and } C' = \frac{t_1t_2}{t_2 - t_1}(L_1 - L_2) \tag{4}$$

L_A' and C′ obtained by the short or the two-point method are primed to distinguish them from the constants obtained by the method of least squares. It has been found convenient to fix the rest periods t_1 and t_2 at 10 and 75 minutes. Under these conditions equations 3 and 4 simplify to

$$L_A' = \frac{75\,L_2 - 10\,L_1}{65} \tag{5}$$

$$C' = 11.54\,(L_1 - L_2) \tag{6}$$

With the aid of equation 5 or 6, or tables based on these equations,

a laboratory technician can readily characterize the properties of a dough from two extensograms (preferably in duplicate).

Initial Comparison of Structural Relaxation Parameters Obtained with the Standard and the Short Methods

Every simplified method must necessarily sacrifice something of the accuracy of the original. To determine how well the structural relaxation parameters C and L_A obtained by the standard procedure may be predicted by the short method, preliminary information was obtained from miscellaneous data available in the laboratory. The data used were culled from another project (1) and represented four different types of flour from Canadian spring wheat, a range of bromate concentrations from 0 to 30 p.p.m., a range of iodate concentrations from 0 to 15 p.p.m., and a range of reaction times from 0 to 4 hours. The tests were made on doughs mixed in an atmosphere of nitrogen.

The structural relaxation parameters C and L_A based on six experimental points for each structural relaxation curve and calculated by the method of least squares were already available. Then only two values, at relaxation times of 10 and 75 minutes, were used to calculate C' and L_A' by the simplified procedure.

Figure 1, left, is a scatter diagram for the relaxation constant C by the two methods. C covers a range from approximately 1000 to 6500. The regression equation shown as the solid line is $C = 0.94\,C' + 8$. The standard error of estimate shown by the broken lines is 330 and the correlation coefficient r is 0.96. It appears then that the short method may well be used instead of the original method, in the range of C values shown. This range would be expected to include most doughs ordinarily encountered. At high values of C, i.e. for doughs containing large amounts of improver and given long reaction times, the relationship was found to deviate from linearity.

Figure 1, right, is a scatter diagram for the constant L_A by the two methods. L_A covers the range from approximately 75 to 250. The regression equation is $L_A = 0.94\,L_A' + 13$. The standard error of estimate is 8.4 and the correlation coefficient $r = 0.98$. The short method for L_A values seems to be even more satisfactory than that for C values.

Mathematically, both the relaxation constant C and the asymptotic load L_A are required to define the structural relaxation completely. However, previous work appeared to indicate that essentially the same information was obtained from either parameter; i.e., the parameters appeared to be associated. The foregoing data were used to obtain an estimate of the extent of this association statistically. The correlation

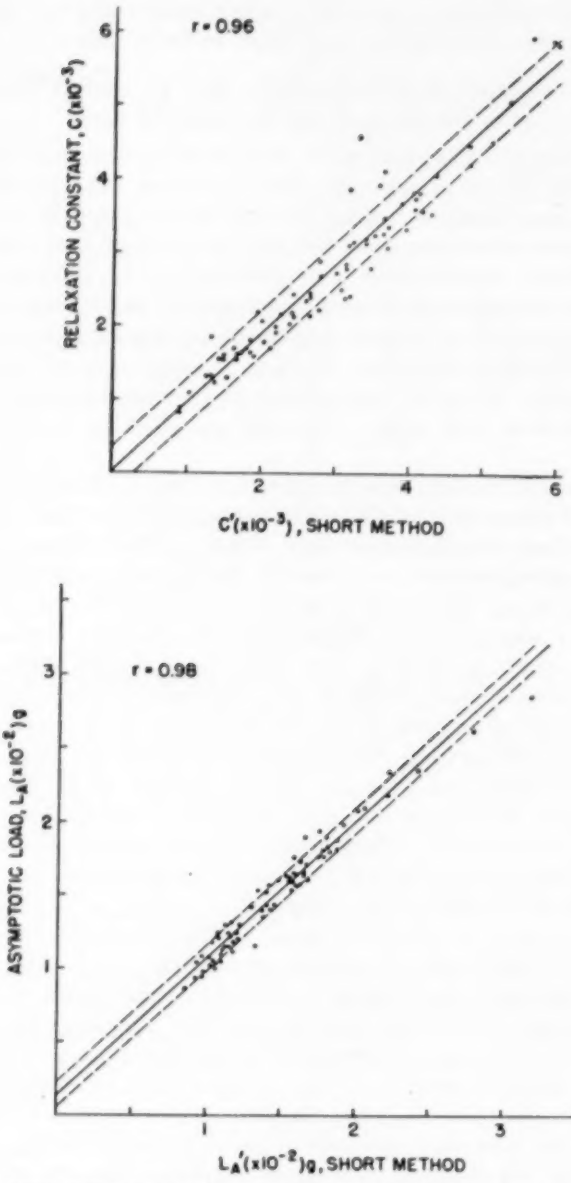

Fig. 1. Scatter diagram for the relaxation constant C (top) and for asymptotic load La (bottom) comparing the original and the simplified methods, for a variety of conditions.

between C and L_A was found to be $r = 0.40$. This value is not high but was obtained from 73 points and was found to be statistically significant at the 1% level. The value of r^2 may be interpreted according to accepted statistical concepts to indicate that the two parameters measure a common property of the dough to the extent of 16% of the total variation. Beyond this, each measures a different set of dough properties. To characterize these properties it is thus necessary to obtain both parameters.

Comparison of the Short Method as a Routine Test with the Regular Procedure for Structural Relaxation

For routine use of the short method for structural relaxation in dough testing, it is desirable to simplify the test as well as to anticipate the practical conditions as much as possible. The data presented in this section were designed to compare a somewhat standardized simplified procedure with the regular procedure.

The range of flours used in the study included six straight-grade, experimentally milled, untreated flours, and five basic types of commercial flour that had been bleached and treated at the mill. All were milled from Canadian hard red spring wheat.

To approach practical conditions in a routine laboratory, all doughs were mixed in air for 2.5 minutes in a G.R.L. mixer with an open bowl, in contrast to doughs mixed under nitrogen in the previous set. Farinograph absorption at a consistency of 540 B.U. was used. For the simplified procedure separate doughs were tested in duplicate on the extensograph at rest periods of 10 and 75 minutes, and the relaxation parameters C and L_A were calculated by substitution in the formulas. The same doughs were also tested by the regular procedure after rest periods of 5, 10, 25, 45, 75, and 105 minutes, and the relaxation parameters were statistically evaluated by the method of least squares.

It has been amply demonstrated in previous publications that the structural relaxation parameters may change markedly with reaction time. Reaction time is operationally defined as the interval between mixing the dough and shaping the test piece. During this time chemical changes may take place in the dough, e.g., bromate reaction. Reaction time should be clearly distinguished from rest period which is the interval between shaping and stretching the dough. During this period the physical process of structural relaxation takes place. The rate of this relaxation describes the dough properties at the end of the reaction time.

Because the properties of dough change with reaction time, the structural relaxation parameters C and L_A for doughs from flours

described in this section were obtained at two different reaction times. To characterize the initial properties of the dough, a reaction time of only 5 minutes was given. To characterize the subsequent change of dough properties with time, a reaction time of 3 hours, corresponding to the molding stage in the baking test, was adopted. At this reaction time untreated flours were also tested with 20 p.p.m. bromate to assess their response to the improver. Two points were obtained for the treated, and three for the untreated flours. Figure 2, which shows the change of C and L_A with reaction time, illustrates the main points at which this series of flours was tested. These points are shown as filled circles at the beginning and the end of each curve.

Figure 3 summarizes the data as scatter diagrams plotting the relaxation constant C and the asymptotic load L_A by the simplified and by the regular procedures. All the data were treated as one population.

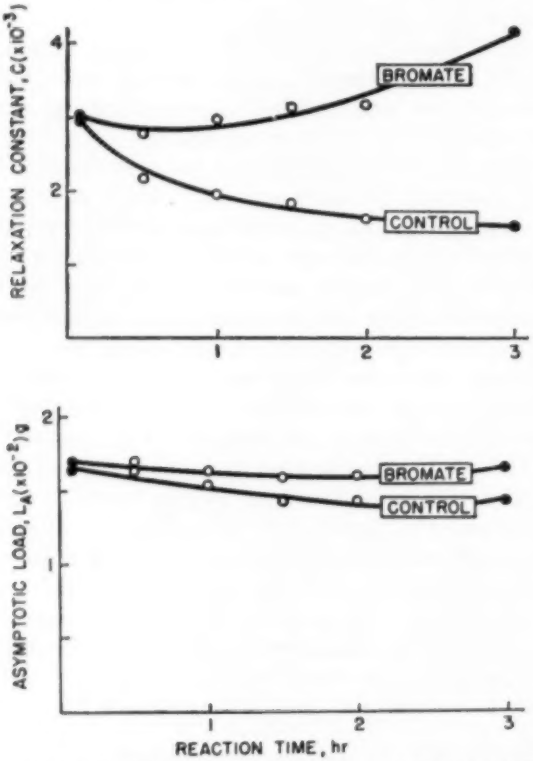

Fig. 2. Change in the relaxation constant C (top) and the asymptotic load L_A (bottom) with reaction time. The filled circles illustrate the initial properties of dough, and the properties after a reaction time of 3 hours for untreated doughs and for doughs containing 20 p.p.m. bromate.

The commercial flours are shown as filled circles for the 5-minute reaction time and as half-open circles for the 3-hour reaction time. The remaining flours were not differentiated.

The regression equation for the relaxation constant C by the regular

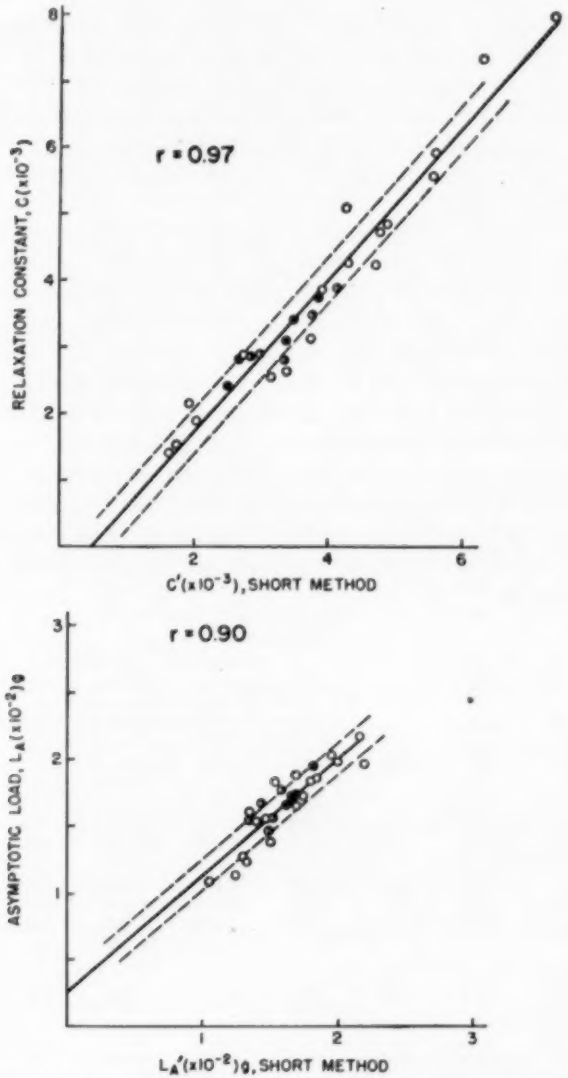

Fig. 3. Scatter diagram for the relaxation constant C (top) and the asymptotic load L_A (bottom) comparing the short method as a routine test with the regular procedure for structural relaxation in dough.

procedure in terms of C′ by the simplified method was found to be $C = 1.15\,C' - 59$ over the range of C values from 1000 to 8000. The standard error of prediction was S.E. = 370 and the correlation coefficient was 0.97. These values are very nearly the same as those obtained in the previous series.

The regression equation for the asymptotic load L_A by the regular procedure and L_A' by the simplified method was found to be $L_A = 0.88\,L_A' + 25$ over the range of L_A values from 100 to 225. The standard error of prediction was S.E. = 12 and the correlation coefficient was 0.90. The asymptotic load data show a somewhat higher error of estimate and a lower correlation coefficient than the data obtained with the larger series.

It is of some interest to examine the place of the commercial flours in this experimental series. The data for the five improver-treated commercial flours and two representative experimental untreated flours are summarized in Table I. The relaxation parameters were calculated by the simplified method. The values for the relaxation constant C′ and for the asymptotic load L_A' lie in the middle of the range shown in Fig. 3. The mean value for the 3-hour relaxation constant for the five commercial flours is somewhat higher than the mean value for the five-minute relaxation constant. In contrast, the untreated flours showed a considerable drop in the relaxation constant after a reaction time of 3 hours. With 20 p.p.m. bromate the C′ values increased considerably. Somewhat analogous changes are indicated by the values for the asymptotic load.

TABLE I

STRUCTURAL RELAXATION DATA OBTAINED BY THE SIMPLIFIED METHOD FOR COMMERCIAL IMPROVER TREATED, AND EXPERIMENTAL UNTREATED FLOUR

Flour	Protein	Ash	Corrected Absorption	Relaxation Constant C′		Asymptotic Load L_A'	
				5 minutes	3 hours	5 minutes	3 hours
	%	%	%				
Commercial, treated							
Top patent	11.8	0.41	60.2	3,860	3,750	184	160
Long patent	12.5	0.51	61.6	3,480	2,860	164	152
Intermediate (cut str.)	12.3	0.47	61.3	3,380	3,000	170	150
Fancy clear	13.4	0.61	61.4	2,710	4,130	169	145
Straight grade	12.8	0.55	62.3	2,520	3,350	168	135
Mean				3,190	3,420	171	148
Experimental							
Untreated	12.4	0.40	62.1	3,920	1,760	183	148
20 p.p.m. potassium bromate	...	...	...	...	4,790	...	201
Untreated	13.0	0.56	61.7	4,350	2,080	170	130
20 p.p.m. potassium bromate	...	...	...	...	5,580	...	184

Discussion

The gain in the use of the simplified method is the ease of calculation of the structural relaxation parameters; the number of tests is reduced to four for the duplicate extensograms at 0 and 75-minute rest periods in contrast to six necessary for the regular procedure. There is only a small sacrifice in the accuracy of the data, although, as has already been pointed out, the short method does not seem to predict the relaxation parameters with adequate precision at high levels of improvers and long reaction times. This range, however, is of experimental rather than of practical interest.

In the traditional method at least four extensograms are usually required to characterize a flour, at a rest period of 45 minutes without allowing a reaction time, and at a rest period of 45 minutes after a reaction time of 135 minutes. The number of extensograms that would be required by the short structural relaxation method is the same for treated flour. Two additional extensograms would be advisable for untreated flour given a reaction time of 3 hours. Reuse of the same doughs for different tests is not favored for either system of testing. The longest time required by the structural relaxation is longer by slightly more than an hour, but tests of this type are conveniently planned to span the noon hour.

In summary, it is considered that the method of describing dough properties in terms of two structural relaxation parameters has now been adequately simplified for routine use in control laboratories. The next stage, of assessing the usefulness of the structural relaxation method over a broad range of practical laboratory experience, will thus now become possible.

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A CALCULATION OF THE SHAPE OF THE ALVEOGRAMS OF SOME RHEOLOGICAL MODEL SUBSTANCES[1]

A. H. Bloksma

ABSTRACT

Using some simplifying assumptions, alveograms of viscous, viscoelastic, and plastic substances have been calculated. The alveogram of a Newton body shows a sharp maximum. Maxwell bodies give less sharp maxima. With increasing relaxation time and constant viscosity of the Maxwell body the height of the maximum decreases. Irrespective of the viscosity, the volume of the dough bubble at which the maximum curve height is reached increases with increasing relaxation time. The alveogram of a Bingham body is obtained by adding to the alveogram of a Newton body a term referring to the yield value; in the case of dough the yield value probably is too small to give a perceptible contribution to the height of the alveogram. Rupture is left out of consideration.

The Chopin Alveograph is commonly used for dough testing, especially in Europe. Nevertheless, remarkably little understanding has been obtained as to the connection between the shape of the alveogram and the rheological properties of the dough. Scott Blair and Potel (7) found the maximum height of the alveogram to be a measure of the viscosity; the maximum volume of the dough bubble was concluded to be a complex function of the viscosity and the elasticity. Hlynka and Barth (4) transformed alveograms into stress-strain curves. These curves, however, can only be understood correctly if the rate of deformation is defined. This cannot be done unequivocally with the alveograph; in the dough bubble the rate of deformation is strongly dependent upon the position on the bubble, as will be shown later. These authors characterize the alveogram by means of one figure, namely, the so-called resistance. Doing so, they use only part of the information that is contained in the curve.

The present author follows the reverse way as compared with that of Hlynka and Barth. He starts by assuming a material having defined rheological properties. Then he tries to derive the form of the alveogram this material would yield. In order to facilitate the calculations, four simplifying assumptions are made:

1. The dough bubble remains part of a sphere until rupture occurs.

2. The volume, V, of the dough bubble increases steadily. This can be written as

$$V = Qt \qquad (1)$$

in which Q is a constant and t is the time. Deviations caused by the

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changing pressure head on the flowing water are neglected. The varying density of the air in the dough bubble is the cause of a smaller deviation (4) that is likewise neglected.

3. The dough is incompressible.

4. During inflation of the bubble every dough particle constantly moves perpendicularly to the surface of the bubble at that place. This assumption permits, among others, the calculation of the thickness of the wall of the dough bubble at an arbitrary time and place.

All four approximations, and especially 1 and 2, cause the idealized model used in this paper to depart from the real dough bubble in the alveograph. Therefore the resulting graphs may only be interpreted qualitatively.

The complete calculation consists of four steps:

1. Geometrical considerations yield the radius, R, and the volume, V, as dependent upon the variable height, h, of the bubble; the thickness of the wall Δ is also found as dependent upon h and the position on the sphere of the dough element studied.

2. Then the rate of deformation of the dough elements is calculated, using assumption 2.

3. Assuming a rheological equation of state the tension in the wall of the dough bubble is calculated.

4. From the tension in the wall, the thickness of the wall, and the radius of the bubble, the pressure in the bubble is calculated.

Geometry

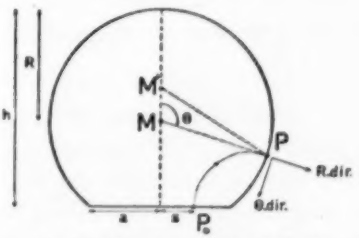

Fig. 1. Geometry of the dough bubble of the alveograph.

Simple geometrical considerations (cf. Fig. 1) yield the radius R and the volume V of the dough bubble as dependent upon the variable height h of the bubble and the constant radius a of its base:

$$R = \frac{h^2 + a^2}{2h} \qquad (2).$$

$$V = \frac{\pi}{6} \cdot h\,(h^2 + 3a^2) \qquad (3).$$

Logically, all quantities that change in time should be calculated as functions of the independent variables t or V. For convenience, however, both V and the other quantities are calculated as functions of the parameter h. Only at the graphical representation of the results is this parameter eliminated.

Assumption 4 permits the prediction of the fate of an arbitrary dough element during inflation. Every dough element may be characterized during the complete process by the constant parameter, s, which represents the distance between its original position, P_0, and the center of the base. Its position, P, at an arbitrary stage of inflation is characterized by the angle θ; the appendix shows how θ is dependent upon the parameters h and s, of which only h changes with time. The increase of the distance between two neighboring elements is a measure of the increase of the surface at the part of the sphere concerned. By means of the assumed incompressibility the thickness of the wall can be found:

$$\Delta = \Delta_0 \left(\frac{a^4 + s^2h^2}{a^2 (a^2 + h^2)} \right)^2 \qquad (4);$$

Δ_0 is the thickness of the original sheet of dough. Equation 4 shows that the thickness of the dough bubble continually increases from the top ($s = 0$) to the base ($s = a$); near the latter Δ approaches Δ_0. This conclusion deviates from the assumption of uniform wall thickness made by Hlynka and Barth (4). The latter assumption calls for a tangential transport of material along the surface of the dough bubble. The present author does not see any cause for this transport. Therefore his assumption 4 seems to be physically more sound. Moreover, according to his experience the wall of the bubble is always thinner at the top than near the base.

Rate of Deformation

A dough element at P is stretched into two directions, namely, the θ-direction (cf. Fig. 1) and the ϵ-direction perpendicular to the plane of drawing. At the same time it contracts along the R-direction. The rate of deformation in the distinct directions is defined as the relative increase of a given length per unit time; it is represented by $\dot{e}$ carrying an index indicating the direction concerned. The rate of deformation in the R-direction is calculated easily, starting from equations 1, 3, and 4:

$$\dot{e}_R \equiv \frac{1}{\Delta} \cdot \frac{d\Delta}{dt} = \frac{1}{\Delta} \cdot \frac{d\Delta}{dh} \cdot \frac{\frac{dV}{dt}}{\frac{dV}{dh}} = -2\dot{e} \qquad (5)$$

in which

$$\dot{e} \equiv \frac{4}{\pi} \cdot Q \cdot \frac{a^2 h\,(a^2 - s^2)}{(a^2 + h^2)^2\,(a^4 + s^2 h^2)} \qquad (6).$$

In a similar way the two rates of stretching are calculated. In agreement with the spherical form and the incompressibility of the material one finds:

$$\dot{e}_\theta = \dot{e}_\epsilon = \dot{e} \qquad (7).$$

Table of Constants Used in the Calculations

a	$= 2.762$	cm.
a^3	$= 21.07$	cm.^{3}
Δ_0	$= 0.25$	cm.
Q	$= 27.0$	cm^3/sec.
$\frac{96\Delta_0 Q}{\pi a^4}$	$= 3.54$	sec.$^{-1}$

The quantity $-\dot{e}_R$ may be directly compared with the rate of increase in height of a rising dough. The former has its maximum value when $s = 0$ and $V/a^3 = 1.007$; using the data from the table of constants, above, one finds for this value 0.53 sec^{-1}. On the other hand, a dough increasing in height from 4 to 10 cm. during one hour shows a mean rate of elongation of

$$\frac{1}{3600} \cdot \ln \frac{10}{4} = 3 \times 10^{-4}\ \text{sec}^{-1}.$$

It is estimated that during oven spring rates of elongation of 5×10^{-4} sec^{-1} occur near the top of the loaf. It is surprising that in the alveograph which is used in assessing the baking value of flours the rate of deformation is up to about 1000 times larger than in the bakery.

Tension in the Wall

The deformation of the dough element at P, described in the preceding section, is caused by tensions in the θ- and ξ-directions. In the case of a *Newton* body with viscosity η the state of tension is described by the following equations provided the R-, θ-, and η-directions are the principal axes of stress (8):

$$p_R + p_h = 2\eta \dot{e}_R$$

$$p_\theta + p_h = 2\eta \dot{e}_\theta$$

$$p_\xi + p_h = 2\eta \dot{e}_\xi$$

in which the hydrostatic pressure p_h is defined by

$$p_h = -\tfrac{1}{3}\,(p_R + p_\theta + p_\xi).$$

Upon substitution of $\dot{e}_R$, $\dot{e}_\theta$, and $\dot{e}_\epsilon$ from equations 5 and 7 one finds:

$$p_R = 0;\ p_\bullet = p_\xi = 6\eta\dot{e} \qquad (8).$$

For a *Maxwell* body (spring and dashpot in series) with a time of relaxation equal to τ and a shear modulus η/τ one may write by analogy (5):

$$p_\bullet = p_\xi = \frac{6\eta}{\tau} \cdot \exp\left(-\frac{t}{\tau}\right) \cdot \int_0^t \dot{e}(t') \cdot \exp\left(\frac{t'}{\tau}\right) \cdot dt'^{2} \qquad (9)$$

in which $t = V/Q$ and $t' = V'/Q$.

Finally, for a *Bingham* body with a fluidity $1/\eta$ and a yield value p_0, the relation is:

$$p_\bullet = p_\xi = 6\eta\dot{e} + p_0 \qquad (10).$$

In this equation p_0 is the yield value of simple tension which is $\sqrt{3}$ times the yield stress in a state of pure shear according to the Mises-Hencky flow condition (3).

Pressure in the Bubble

The relation between the pressure P in a bubble, its radius R, the thickness Δ of the wall and the tension p in the wall can be shown to be

$$P = 2p \cdot \frac{\Delta}{R} \qquad (11).$$

In this equation p is identical with p_θ and p_ϵ from the preceding equations. In the derivation of equation 11, Δ is assumed to be small compared with R. Moreover, the dependence of the thickness Δ upon the position on the sphere as described by equation 4 is neglected. Equation 11 is made plausible when one remembers that the two halves of the sphere are pushed one from the other by the pressure P on a surface πR^2; the two halves are held together against this pressure by the tension p working on a surface $2\pi R\Delta$. Setting $P \times \pi R^2$ equal to $p \times 2\pi R\Delta$ one obtains equation 11.

Combining equations 1 to 4, 6, and 8 to 11 one obtains the following expressions for the pressure P. They consist of a factor that has the dimension of a pressure and that does not depend upon the time, and a dimensionless factor F which is a function of the dimensionless quantities V/a^3, $Q\tau/a^3$ and s/a.

Newton body:

$$P = \frac{96\eta\Delta_0 Q}{\pi a^4} \cdot F_1 \qquad (12).$$

[2] t' Indicates that $\dot{e}$ represents the rate of deformation at time t' and bubble height h'.

$$F_1 \equiv a^2 (a^2 - s^2) \cdot \frac{h^2 (a^4 + s^2 h^2)}{(a^2 + h^2)^3} \tag{13}$$

Maxwell body:

$$P = \frac{96 \eta \Delta_0 Q}{\pi a^4} \cdot F_2 \tag{14}$$

$$F_2 \equiv \frac{\pi}{2 Q_\tau} \cdot a^2 (a^2 - s^2) \cdot \frac{h (a^4 + s^2 h^2)^2}{(a^2 + h^2)^3} \cdot \exp\left(-\frac{V}{Q_\tau}\right) \times \int_0^h \frac{h'}{(a^2 + h'^2)(a^4 + s^2 h'^2)} \cdot \exp\left(\frac{V'}{Q_\tau}\right) \cdot dh' \tag{15}$$

Bingham body:

$$P = \frac{96 \eta \Delta_0 Q}{\pi a^4} \cdot F_1 + \frac{4 p_0 \Delta_0}{a} \cdot F_3 \tag{16}$$

$$F_3 \equiv \frac{h (a^4 + s^2 h^2)^2}{a^3 (a^2 + h^2)^3} \tag{17}$$

In Figs. 2 to 4 the functions F are plotted against V/a^3 for different values of s/a and $-$ in the case of $F_2 - Q_\tau/a^3$. The integral in equation 15 was approximated numerically. The values of s/a have been chosen in order that imaginary rings characterized by these values would divide the total material into four equal parts. For an illustration of the significance of the dimensionless abscissa values the first two graphs (top, Fig. 2) are provided with a volume and a time scale, respectively, at the top; these scales hold under the conditions given in the table of constants. Under the same conditions the significance of the values chosen for Q_τ/a^3 can be read from the left-hand part of Table I.

Discussion

Figure 2 shows that for Newton and Maxwell bodies the shape of the curve strongly depends upon s/a. It is physically absurd, however, that the pressure in the bubble should be dependent upon the place on the surface. Therefore assumption 1, that the dough bubble remains part of a sphere, cannot be correct for these bodies. In spite of the crude approximations involved in the calculations, the graphs enable us to draw conclusions as to the shape of the alveogram to be expected for these rheological model substances. The correct alveogram will show a certain resemblance to some mean of the curves drawn.

A Newton body may be considered as a Maxwell body with zero relaxation time. Because of the similarity between equations 12 and 14 the functions F_1 and F_2 can be compared directly. Figure 2 permits a comparison of the shape of the curves for a Newton and three Maxwell

bodies. The different parts of Fig. 2 have different vertical scales. For comparison of the height of the maxima, four curves with identical s/a and different relaxation times are taken together in Fig. 3.

The sharp maximum in the case of a Newton body with constant viscosity is a consequence of the high rate of deformation occurring when V/a^3 is equal to about unity. However, one should realize that the assumption of a steady increase of the bubble volume tends to exaggerate the sharpness of the peak. As the volume increases the pressure decreases sharply because the rate of deformation decreases, the radius of the sphere increases, and the wall becomes thinner (cf. eq. 11). Maxwell bodies show peaks that are lower and less sharp; moreover, the maximum is reached at a later moment (cf. Table I). The deviation from the behavior of the Newton body increases with increasing relaxa-

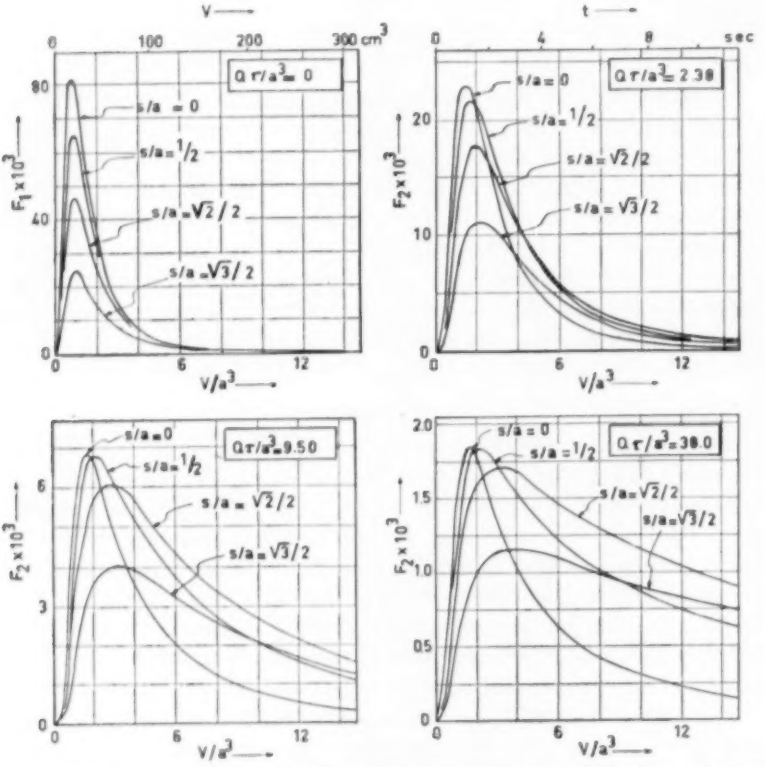

Fig. 2. Theoretical alveograms for Newton and Maxwell bodies. The upper left-hand part refers to a Newton body. The other parts hold for Maxwell bodies with different relaxation times, τ. The value s/a denotes the position on the surface of the bubble. The curves for s/a = 1 coincide with the abscissae in all four parts.

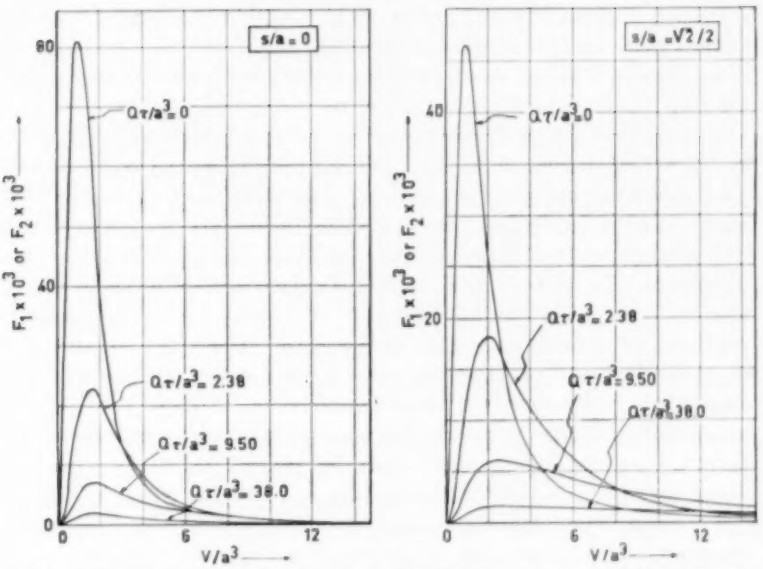

Fig. 3. Theoretical alveograms for Newton and Maxwell bodies. The curves of Fig. 2 have been arranged in another way in order to demonstrate the influence of the relaxation time on the curve height.

tion time. In the Maxwell body the elastic component receives a part of the rapid deformation that causes the sharp maximum in the case of the Newton body. The flow of the viscous element proceeds at a more uniform rate. Consequently the dissipation of energy as measured by the area under the curve is smaller.

The conclusion of Scott Blair and Potel (7) that the maximum height of the curve is a measure of the viscosity does not hold for Maxwell bodies; equation 14 and Fig. 3 show that this height decreases at

TABLE I

INFLUENCE OF THE RELAXATION TIME ON THE MAXIMUM CURVE HEIGHT AND THE VOLUME OF THE BUBBLE AT WHICH THE MAXIMUM IS ATTAINED

RELAXATION TIME			MAXIMUM CURVE HEIGHT[a]		VOLUME AT WHICH THE MAXIMUM CURVE HEIGHT IS ATTAINED[a]		RATIO OF THE CURVE HEIGHT AT 100 cm[b] AND THE MAXIMUM CURVE HEIGHT[a]
τ[b] sec	Qτ[b] cm³	Qτ/a³	$F_1 \times 10^3$ or $F_2 \times 10^3$	P[c] g/cm²	V/a³	V[b] cm³	P_{100}/P^b
0.0	0	0.0	46.	83	1.0	21	0.10
1.86	50	2.38	18.	32	2.0	43	0.51
7.42	200	9.50	6.1	11	2.6	54	0.84
29.7	800	38.0	1.7	3	2.9	62	0.94

[a] The curves for which s/a = √2/2 have been considered representative for the correct alveogram.
[b] Calculated using the values in the table of constants.
[c] Calculated using the values in the table of constants and an assumed viscosity of $\eta = 5 \times 10^6$ g.cm.$^{-1}$ sec.$^{-1}$

increasing relaxation time in spite of the viscosity's remaining constant. Table I gives an idea of the magnitude of this effect; as a consequence of the idealized model used, the figures cannot be interpreted quantitatively.

The curves of Figs. 2 and 3 confirm the conclusions of Hlynka and Barth (4) that the maximum in the normal alveogram need not be a consequence of changing rheological properties of the material. All models used in this paper have constant rheological properties; nevertheless, their curves show the normal shape of an alveogram. The maxima in these curves are caused by the geometry of the experiment. Therefore no physical reason exists for characterizing an alveogram by means of the maximum height of the curve. The author agrees with Hlynka and Barth (4) that the curve height at an arbitrarily chosen volume is a better measure. However, he wants to suggest as a second measure the volume at which the maximum curve height occurs because this volume is dependent upon the relaxation time, provided the dough behaves as a Maxwell body; Halton and Scott Blair (1, 2) found that a good bread dough has, among other characteristics, a long relaxation time. The ratios of the height of the curve at a volume of, for instance, 100 cm.^{3} or just before rupture occurs and the height of the maximum might also be useful for characterizing the alveogram. These ratios also depend upon the relaxation time (cf. Table I) and can be measured more accurately than the volume at the maximum curve height.

If the dough behaves as a Bingham body its yield value contributes only perceptibly to the height of the curve if the second term of the right-hand side of equation 16 is sufficiently large. From a comparison of Figs. 2, upper left, and 4, together with the data from the table of constants, it is concluded that the condition for a noticeable contribution of the yield value to the curve height is $p_o/\eta > 3 \times 10^{-3}$ sec^{-1}. Data of Schofield and Scott Blair (6) suggest that the quotient p_o/η for a bread dough is of the order of 3×10^{-4} sec^{-1}. Therefore it is considered improbable that at the given rate of inflation the yield value of the dough contributes perceptibly to the curve height.

The treatment given might be extended by considering other rheological models. As dough shows retarded elasticity the Kelvin body (a spring and dashpot parallel) is worthy of consideration. However, unless the compliance of the elastic element is very high and the model approaches a Newton body, it yields an alveogram with a maximum followed by a minimum. Until now only models with one or two parameters have been considered. As a matter of fact the description of the rheological behavior of dough asks for more than two param-

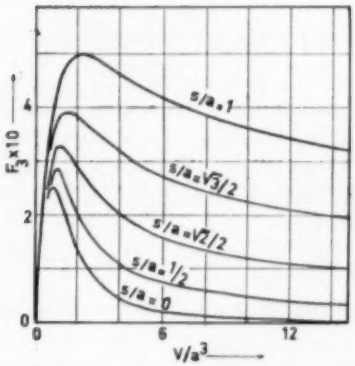

Fig. 4. The contribution of the yield value of a Bingham body to the height of the alveogram. The value s/a denotes the position on the surface of the bubble.

eters. At the expense of more elaborate calculations a model with more parameters undoubtedly permits a closer approach of the experimental alveograms. Such refinements, however, will have limited significance unless assumption 1 about the spherical form of the dough bubble has been replaced by an approach that is more satisfactory from a physical point of view.

The phenomenon of rupture not being introduced into this treatment, the theory cannot predict the maximum volume of the dough bubble to be attained.

Appendix

The change of the angle θ during inflation is a consequence of the rise of the center of the dough bubble. Say that the center proceeds from M to M′ when the height h increases by dh. The distance MM′ is calculated from equation 2:

$$MM' = dh - dR = \frac{h^2 + a^2}{2h^2} \cdot dh \qquad (18).$$

Application of the sine rule in $\Delta MPM'$ yields:

$$\frac{MM'}{\sin\, d\theta} = \frac{R}{(\sin\, (\theta + d\theta)}$$

which can be simplified after substitution of equations 2 and 18:

$$\frac{d\theta}{\sin\, \theta} = \frac{dh}{h} \qquad (19).$$

Equation 19 is integrated. The integration constant is determined by the condition

$$s = \lim_{h \to 0} R. \sin \theta.$$

The result is

$$\cos\theta = \frac{a^4 - s^2h^2}{a^4 + s^2h^2} \qquad (20).$$

In the original dough sheet the surface between two rings with radius s and s + ds is $2\pi s \cdot ds$. At an arbitrary stage of inflation this surface equals $2\pi R^2 \cdot \sin\theta \cdot d\theta$. Taking the thicknesses of the original sheet and of the wall of the bubble Δ_0 and Δ respectively, the condition of incompressibility yields

$$2\pi s \cdot ds \cdot \Delta_o = 2\pi R^2 \cdot \sin\theta \cdot d\theta \cdot \Delta.$$

Upon substitution of equations 2 and 20 one obtains equation 4.

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A NOTE ON THE BIREFRINGENCE OF STRETCHED GLUTEN[1]

A. H. Bloksma[2] and J. Isings[3]

The change in the birefringence of macromolecular substances during the relaxation of stress at constant strain permits the distinction of some mechanisms of the relaxation process (13, 14, 15, 16, 17). This method may also be applied to gluten. Birefringence of stretched gluten has been reported by Ritter (10, 11). Hanssen (2), however, doubts the birefringence of gluten and explains Ritter's observations by the presence of small starch granules.

A crude wheat gluten was prepared by washing a dough with a 0.07 molar phosphate buffer of pH 6.8. The gluten was solved in acetic acid and purified according to Lusena's method (8); the precipitation and second dispersion were omitted. It was lyophilized in an apparatus as described by Pratt and Yamaguchi (9). Another sample of an isolated protein fraction (Zwickelprotein) having gluten-forming properties was obtained according to Hess (3, 4, 5); it was purified as described above. Microscopical investigation of the preparations stained by means of iodine showed the starch content to be less than 1%. The protein was mixed with an appropriate amount of water and left undisturbed for at least 15 minutes. Then a portion of it was stretched to about ten times its length, resulting in a translucent layer. This was clamped between an object slide and a cover-glass. The optical path difference was measured by means of a Zeiss-Winkel polarizing microscope with a Sénarmont compensator. The birefringence was calculated from the optical path difference and the thickness of the layer; the latter was estimated by means of a micrometer.

A positive birefringence of the order of 0.4 $m\mu$ optical path difference per μ thickness was observed. Figure 1 shows its decrease on standing at constant deformation.

Our results confirm Ritter's observations. Unlike Hanssen we do not see any reason to ascribe the observed birefringence to starch. An explanation along this line would demand, among other things, the presence of a considerable amount of starch granules with diameters less than the resolving power of the objective used (0.1 μ), but there is no indication of this. Moreover, birefringence caused by starch would not be expected to decrease with time.

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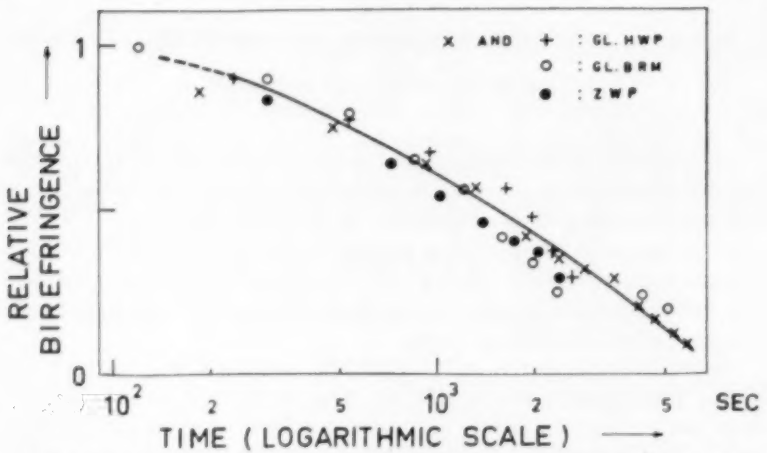

Fig. 1. The decrease of birefringence in the course of time at constant deformation. The birefringence is given in an arbitrary scale, its value at the first observation taken equal to unity. The time is measured from the first observation. The different signs refer to different experiments with three proteins, namely, gluten from hard wheat patent flour (GL. HWP), gluten from a breadmaking flour of about 75% extraction (GL. BRM), and Zwickelprotein (ZWP). Temperature, 22° C.

The decrease of birefringence with time is similar to the relaxation of stress in gluten and dough (1, 6, 7, 12); perhaps it proceeds more slowly (18). The birefringence shows that the disorder in the arrangement of the peptide chains increases during relaxation. Obviously the relaxation of stress is caused by a chemical reaction or viscous flow (15); the interchange of cross links is the most probable of the chemical reactions in the case of gluten. The present experiments indicate that crystallization and orientation of crystalline areas are negligible during relaxation.

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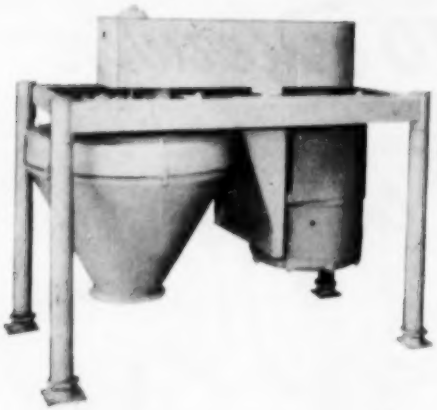

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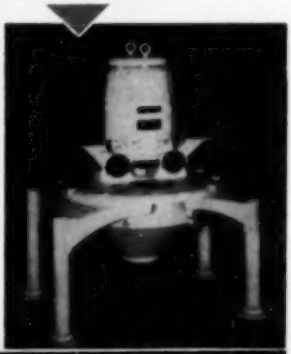

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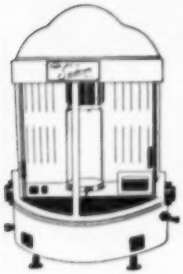

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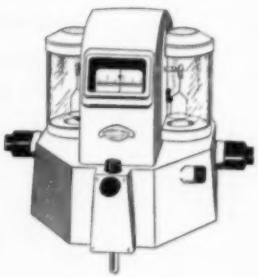

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Gluten varies in quality and quantity. Some is difficult to condition, but most of it, when properly mellowed, can be made to take up additional moisture. Paniplus assures Proper Gluten Development and Maximum Hydration.

THE Paniplus COMPANY

4049 Penn., Kansas City, Mo.

526 Bay Street, Toronto, Canada

Atkinson Milling Co. of Minneapolis, Minn.,
where Wallace & Tiernan flour treatment is used

HOW ATKINSON MILLING COMPANY USES W&T FLOUR TREATMENT

The Atkinson Milling Company regularly processes up to 6000 cwt. of flour daily. To insure that the flour is uniformly aged, properly enriched, and of the best color, this progressive mill uses Wallace & Tiernan flour treatment. This includes: Dyox® for maturing; a Beta-Chlora® unit for conditioning; Novadelox® for bleaching; and N-Richment-A® for enrichment. In addition, the Atkinson Milling Company has available, at any time, skilled and experienced technical assistance from Wallace & Tiernan to aid them in the solution of their flour processing problems.

The Atkinson Milling Company is only one of the many flour milling companies using W&T Flour Treatment. If your mill is not one of these, investigate the advantages of Wallace & Tiernan's complete flour service.

NOVADEL FLOUR SERVICE DIVISION
WALLACE & TIERNAN INCORPORATED
25 MAIN STREET, BELLEVILLE 9, NEW JERSEY
REPRESENTATIVES IN PRINCIPAL CITIES

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